VOLUME 86 NUMBER 4

UNIVERSITY ... AND ...

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

April, 1950

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PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

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BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

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This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

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THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorised on July 3, 1918

THE EFFECT OF THE SIZE OF THE INOCULUM (NUMBER OF TREPONEMES) UPON THE COURSE OF EXPERIMENTAL SYPHILIS IN THE RABBIT

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Received for publication, January 17, 1950

Chesney and Schipper (1) have recently reported a study designed to determine the effect of the method of inoculation on the course of experimental syphilis in the rabbit. Three methods of inoculation were employed,—intracutaneous, intratesticular and intravenous. The authors found that the total incidence of generalized lesions was greatest in the animals inoculated intravenously, least in the animals inoculated intracutaneously, while in the group inoculated intratesticularly it occupied a position midway between those of the other two groups. Moreover the number of individual rabbits showing generalized lesions varied in the same way depending upon the method of inoculation.

While other observers have compared the course of experimental syphilis in rabbits inoculated in different ways, none have reported studies dealing with all three types of inoculations in animals belonging to one breed and inoculated at one time. Moreover the numbers of animals in the three groups inoculated by Chesney and Schipper in different ways were sufficiently large to be statistically significant. The method of inoculation did not seem to exert any effect upon the site at which the generalized lesions developed.

In the discussion of their findings the authors pointed out that the greater frequency of generalized lesions in the rabbits inoculated intravenously might well have been due either to (a) the presence of a greater number of treponemes in the circulating blood or (b) to a partial protective effect engendered by the development of a cutaneous or testicular syphiloma at the site of inoculation.

In order to test the first of these two hypotheses it seemed desirable to carry out an experiment in which a large number of rabbits

*This study was made possible by a grant from the Public Health Service, National Institute of Health.

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all of one breed would be inoculated intravenously with varying numbers of treponemes. The purpose of this paper is to record the results of such an experiment.

METHOD

Two groups of 50 female chinchilla rabbits each, divided in such a way that they corresponded closely to one another in their individual body weights, were inoculated intravenously with measured amounts of a suspension of a virulent strain of *T. pallidum* (Nichols strain) in a fluid medium used by Dr. Robert A. Nelson (2) for carrying out tests of the motility of *T. pallidum* in the presence of different agents. We are greatly indebted to Dr. Nelson for supplying us with a quantity of this medium sufficient for our use.

The first group of rabbits (Group A) received 770,000 *T. pallida* suspended in 0.1 cc. of fluid, while the second group (Group B) received one one-hundredth of this amount, or 7,700 *T. pallida* also suspended in 0.1 cc. of fluid. Five additional rabbits of varying breeds were inoculated intracutaneously at two sites on the back with the smaller sized inoculum as virus controls. The inoculations were all made on November 2, 1948.

The rabbits were examined for the presence or absence of lesions at weekly intervals by inspection and palpation for a period of 24 weeks. No radiographic examinations were performed. At the end of the period of observation the popliteal lymph nodes and a portion of the liver were removed from those animals which had failed to show any manifestations of infection and were emulsified separately in physiological salt solution. These emulsions were then inoculated intratesticularly into normal animals. In every instance the node emulsions and the liver emulsions were inoculated into two animals each. The animals to which these tissue transfers were made were observed for at least 134 days before being discarded as negative.

RESULTS

Group A, larger inoculum (770,000 T. pallida). Of the 50 animals in this group at the outset of the experiment 45 survived for the entire period of observation, namely, 163 days. The occurrence and distribution of syphilitic lesions in these animals is shown in Table 1, in which the rabbits are arranged in the order of their body weights. It will be seen that in 39 (87%) of the 45 animals there developed one or more lesions of syphilis detectable by palpation or inspection. The average number of lesions observed per rabbit was 6. Skin lesions were twice as numerous as bone lesions although there were approximately the same number of animals showing both types of lesions. No eye lesions were noted save those involving the lids. The incubation period of the first

lesion varied from 6 to 23 weeks, the average being 11.8 weeks. The total number of lesions of all kinds which developed in the animals of this group was 232.

Group B, smaller inoculum (7,700 T. pallida). Of the 50 animals in this group inoculated at the outset of the experiment 42 survived for the entire period of observation. The occurrence and distribution of syphilitic lesions in these animals is shown in Table 2, in which likewise the rabbits are arranged in the order of their body weights. It will be seen that in 20 (48%) of these animals there developed one or more lesions of syphilis detectable by palpation or inspection, that again skin lesions were twice as numerous as bone lesions although in approximately the same number of rabbits there developed both types of lesions, and that again no eye lesions were noted save those involving the lids. The average number of lesions observed per rabbit was 7. The incubation period of the first lesions developing in the animals of this group ranged from 8 to 23 weeks, with an average of 13.4 weeks. The total number of lesions of all kinds which developed in the animals of this group was 148.

In the control animals inoculated intracutaneously on the back with the two inocula of different size, syphilitic lesions developed at the site of inoculation in all but one, an animal in Group A receiving the larger inoculum. Just why this animal failed to show any lesion at the site of inoculation is not clear.

The results of the experiment are summarized in Table 3, in which are included the results of tissue transfer (lymph nodes and liver) from those animals in which no lesions at all were observed. Thus, of the 6 animals in which no lesions were detected following injection with the larger inoculum, 4 or 66 per cent gave positive tissue transfers, while of the 22 animals in which no lesions developed following injection with the smaller inoculum 13, or 60 per cent, yielded positive tissue transfers.

Altogether 43 of the 45 animals receiving the larger inoculum were proved to have been infected, while 33 of the 42 animals receiving the smaller inoculum were proved to have been infected, but the incidence of asymptomatic infection was much higher in the latter group (40%), than in the former (9.3%).

The data presented in Tables 1 and 2 have been reviewed from the

Table 1 Occurrence and Distribution of Generalized Lesions of Syphilis in Rabbits Inoculated Intravenously with 770,000 *Treponemata Pallida*

Rabbit No.	Weight gms.	BONE: Nose Rt.	Nose M	Nose Lt.	Wrist Rt.	Wrist Lt.	Fore foot Rt.	Fore foot Lt.	Hind foot Rt.	Hind foot Lt.	Ankle Rt.	Ankle Lt.	Heel Rt.	Heel Lt.	SKIN: Nose Rt.	Nose Lt.	Ear Rt.	Ear Lt.	Lips Rt.	Lips Lt.	Fore leg Rt.	Fore leg Lt.	Fore foot Rt.	Fore foot Lt.	Hind leg Rt.	Hind leg Lt.	Hind foot Rt.	Hind foot Lt.	Vulva	Anus	Tail	EYE: Lid Rt.	Lid Lt.	Sclera Rt.	Sclera Lt.	Cornea Rt.	Cornea Lt.	Lens Rt.	Lens Lt.	Incubation period Wks.	Total no. of lesions	Result of tissue transfer
8277	1700				+		+	+			+				+												3/7		+	+	+									11	11	
8276	1750	+											+		+												4/7			+										11	9	
8287	1790	+											+													+	4/7				+									11	6	
8254	1850		+										+		+						+	+					+			+	+	+								6	11	
8279	1900	+		+																							+		+	+									9	5	
8359	1920	+		+		+																					4/5	+		+			+							8	8	
8358	1925																																							0	0	Neg.
8275	1970			+									+		+											+	4/5			+										9	7	
8316	1980	+		+	+	+															+	+					4/5			4+5	+	+		+						10	18	
8299	2000		+																				+			+	4/5	+	4/5		+										8	9	
8355	2000																				+					+	+			+										9	4	
8337	2025																																							0	0	Neg.
8353	2080					+																																		13	1	
8307	2120																		4/5			+					+			+		+								12	6	
8254	2120			+		+							+												+					+		+								12	6	
8229	2150																										+													22	1	
8314	2150	+		4/5	+	+				+									+	+					4/5	4/5			+		+	+								12	18	
8319	2175	+		+		+					+		+						+	+					4/5	+			+											9	16	
8306	2200	+			+	+																								+										12	4	
8331	2200																																							0	0	Pos.
8251	2230				+	+																+																		17	3	
8281	2250			+																						+				+										13	2	
8247	2270																													+										15	1	
8272	2270		+																																					9	1	
8309	2270	+																									+													14	2	
8340	2275	+			+	+																																		13	3	
8249	2300														+	4/5																									22	5	
8334	2300		+																									+													13	2	
8330	2300	+			+	+																							4+5											11	5	
8310	2300		+																								4/5													11	3	
8242	2325																																							0	0	Pos.
8311	2375	4/5		+	+												+				+				4/5	+				+	+									8	11	
8320	2400	+		+		+																			+															10	4	
8266	2450														+																									23	1	
8313	2450																												4+5		+									9	4	
8297	2460				+																						+													12	1	
8312	2460	+	+		+	+																					+													8	5	
8287	2470																																							0	0	Pos.
8348	2475	+		+	+	+																																		11	4	
8315	2480	+		4/5	+	+													+		4/5	4/5			4/5	4/5	+			+		+	+								11	24	
8352	2540	+														+														+											8	3	
8252	2540																																							0	0	
8255	2550																										+	+													14	2	
8241	2600															+				+	+	+				4/5															10	7	
8238	2700																														+										12	1	
Total lesions		1	0	5	6	11	1	4	1	1	0	1	2	0	1	1	5	5	5	0	1	1	4	8	10	1	0	2	16	20	38	3		23		19		6	2																		

TABLE 3

Comparison of incidence and distribution of generalized lesions in syphilitic rabbits, inoculated intravenously with inocula of different size

	SIZE OF INOCULUM NUMBERS OF T. PALLIDA	NO. OF ANIMALS OBSERVED	ANIMALS SHOWING LESIONS OF ANY SORT		ANIMALS SHOWING BONE LESIONS		ANIMALS SHOWING SKIN AND M.M. LESIONS		ANIMALS SHOWING EYE LESIONS		ANIMALS SHOWING NO LESIONS		ANIMALS WITH NO LESIONS BUT YIELDING POSITIVE TISSUE TRANSFERS		TOTAL NUMBER OF LESIONS			
			No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	Bone	Skin	Eye	Total
Group A	770,000	45	39	87	30	67	32	71	7	16	6	13	4	66	75	149	8	232
Group B	7,700	42	20	48	15	36	18	43	5	12	22	52	13	60	46	93	9	148

TABLE 4

The effect of body weight on the incidence and distribution of generalized lesions in syphilitic rabbits inoculated intravenously with inocula of different size

	SIZE OF INOCULUM NUMBERS OF T. PALLIDA	NO. OF ANIMALS OBSERVED	ANIMALS SHOWING LESIONS OF ANY SORT		ANIMALS SHOWING BONE LESIONS		ANIMALS SHOWING SKIN AND M.M. LESIONS		ANIMALS SHOWING EYE LESIONS		ANIMALS SHOWING NO LESIONS		ANIMALS WITH NO LESIONS BUT YIELDING POSITIVE TISSUE TRANSFERS		TOTAL NUMBER OF LESIONS			
			No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	Bone	Skin	Eye	Total
Group A	770,000																	
1. Animals weighing less than 2270 gms.		22	19	86	16	72	18	82	5	23	3	14	1	33	42	98	5	145
2. Animals weighing 2270 gms. or more		23	20	87	14	61	14	61	2	9	3	13	3	100	33	51	3	87
Group B	7,700																	
1. Animals weighing less than 2270 gms.		20	12	60	7	35	11	56	4	20	8	40	4	50	25	65	7	97
2. Animals weighing 2270 gms. or more		22	8	36	8	36	7	32	1	5	14	64	9	66	21	28	2	51

standpoint of whether or not any relationship could be demonstrated between the incidence and distribution of syphilitic lesions on the one

hand, and body weight of the rabbits on the other. It was possible to divide each major group into two almost equal subgroups, one containing all the rabbits weighing less than 2270 grams, the other containing all the animals weighing 2270 grams and more. In Table 4 are summarized the data obtained by such an arrangement.

From this table it is seen that the total number of lesions developing in each of the subgroups containing the lighter and presumably younger animals was greater than the number developing in the subgroups containing the heavier and presumably older animals, irrespective of the size of the inoculum. However these differences were due largely to differences in the number of skin lesions developing in the various groups; differences observed in the incidence of bone and eye lesions were too small to be significant. Moreover there were no signifi-

TABLE 5

Comparison of results of lymph node transfer and liver transfer in syphilitic rabbits inoculated intravenously

ANIMALS FROM WHICH TISSUE TRANSFERS WERE MADE	ANIMALS SHOWING POSITIVE NODE TRANSFER ONLY		ANIMALS SHOWING POSITIVE LIVER TRANSFER ONLY		ANIMALS SHOWING POSITIVE NODE AND LIVER TRANSFER		ANIMALS SHOWING NEGATIVE NODE AND LIVER TRANSFER		ANIMALS SHOWING POSITIVE TISSUE TRANSFER	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
27	11	41	0	0	5	18	11	41	16	60

cant differences, as between lighter and heavier animals in the number of animals in which syphilitic lesions of any sort developed.

The results obtained from tissue transfer deserve brief comment at this point. As has been stated previously, both popliteal nodes and portions of liver were transferred from the asymptomatic animals in the series to normal male rabbits by intratesticular inoculation in order to determine if infection had occurred. This procedure was carried out in 27 animals altogether. The results are shown in Table 5.

Of the 27 animals from which tissue was transferred the transfer was positive in 16 instances or 60 per cent. Of these 16, the lymph nodes alone gave a positive result in 11, while both the nodes and the liver yielded positive nodes in 5. In no instance did the liver alone give a positive result. Lymph nodes alone, then, gave twice as many positive transfer results as did nodes plus liver. These results seem to indicate

that the lymph nodes in the syphilitic rabbit constitute a more constant reservoir of the infection than does the liver.

DISCUSSION

The course of syphilitic infection in rabbits inoculated intravenously may be judged by either (a) the number of animals in which lesions develop which are detectable by inspection or palpation, or (b) by the total number of lesions which occur irrespective of the number of animals in which they develop. Judged by either of these criteria the results of the experiment are clear cut. The introduction of a larger inoculum of virulent *T. pallida* into the veins of a group of normal female rabbits all of one breed resulted in more animals showing lesions of the disease than in the case of a comparable group receiving an inoculum 1/100th as large: it also resulted in the occurrence of a greater total number of lesions in the animals receiving the larger inoculum.

The distribution of the lesions was essentially the same in both groups irrespective of the size of the inoculum, as was also the average number of lesions observed per rabbit in those animals showing signs of the infection. Apparently, if lesions appeared in a rabbit in either group the number of such lesions which developed during the course of the infection was independent of the size of the inoculum.

Skin lesions were relatively more numerous in the animals in this experiment than in the series inoculated intravenously by Chesney and Schipper (1), but otherwise the distribution was not greatly different.

It is difficult to draw any definite conclusions in regard to the effect of weight (and therefore presumably age) on the course of the infection in these animals. Such evidence as was forthcoming suggested that in general the lighter animals showed a higher incidence of skin lesions than the heavier ones, irrespective of the size of the inoculum, but such a statement must be made with reservations.

Particular interest attaches, in our opinion, to the fact that although manifest signs of syphilis occurred in only 20, or less than 50 per cent of the animals inoculated intravenously with the smaller inoculum, nevertheless infection could be demonstrated by tissue transfer in 13 of the remaining 22 animals in that group. In other words the smaller inoculum was far behind in producing manifest lesions but not so far

behind in producing actual infection since in the 45 animals inoculated with the larger inoculum 43 were proved to have been infected, whereas in the 42 animals receiving the smaller inoculum as many as 33 were proved to have been infected.

The experiment showed quite clearly that the lymph nodes are a more constant reservoir of syphilitic infection in rabbits than is the liver.

SUMMARY AND CONCLUSIONS

Two groups of female rabbits all belonging to one breed (Chinchilla) were inoculated with different amounts of *T. pallida*. One group received an inoculum containing 770,000 treponemes, the other an inoculum containing 7,700 treponemes. In the 45 animals receiving the larger inoculum there were 39 (87%) which showed lesions recognizable by inspection or palpation, whereas in 42 animals receiving the smaller inoculum lesions were observed in 20 or 48%. The total number of lesions of any sort developing in animals receiving the larger inoculum was 232, as against 148 in the animals receiving the smaller inoculum.

The total number of animals proved to have been infected with the larger inoculum was 43 out of 45 inoculated, whereas the total number proved to have been infected with the smaller inoculum was 33 out of 42 inoculated. The incidence of asymptomatic infections was 40% in the animals receiving the smaller inoculum as against 9.3% in the animals receiving the larger inoculum.

Body weight did not seem to exert any significant effect upon the incidence of animals showing lesions of any sort. However the incidence of skin lesions was greater in the lighter animals (those weighing less than 2,260 gms.) than in the heavier animals (those weighing more than 2,270 gms.).

In the animals in which an asymptomatic infection developed the lymph nodes were found to be infectious more often than the liver, indicating that they are a more constant reservoir of syphilitic infection than that organ.

The experiment certainly suggests that within the limits of the inocula employed the size of the inoculum (that is, total number of treponemes), has a direct effect when injected intravenously upon the course of experimental syphilis in the rabbit, as judged by the number

of animals in which lesions detectable by inspection and palpation develop, also by the total number of such lesions developing during the course of the infection. It offers support, therefore, for the hypothesis that the greater frequency of generalized syphilitic lesions in the rabbit after intravenous inoculation of *Treponema pallidum*, as compared with that after intracutaneous or intratesticular inoculation, is due to a greater number of treponemes reaching the circulating blood. The experiment does not exclude the possibility, however, that other factors such as individual susceptibility or partial immunization engendered by the development of a local lesion at the site of inoculation (skin, testis) may also play a role in the demonstrated relationship between the site of inoculation and the development of generalized syphilitic lesions in the rabbit. The behavior of some of our animals certainly indicated that individual susceptibility of the host is still an important factor in determining the course of experimental syphilis in the rabbit.

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A CARBONYL REACTION DIFFERENTIATING THE FETAL ZONA RETICULARIS OF THE HUMAN ADRENAL CORTEX FROM THE MOUSE X ZONE

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Received for publication Jan. 23, 1950

INTRODUCTION

The functional significance of the remarkable reduction in size of the human adrenal after birth, associated with the degeneration of the fetal zona reticularis, is not well understood. This adrenal involution, originally observed in 1908, has recently been studied by Benner (2). There do not appear to be quantitatively comparable postnatal changes in most mammals, although Hill (10), who reviewed the earlier literature, inferred, on the basis of indirect considerations, that there was an involution in other primates comparable to that in the human.

The involution of the X zone of the mouse adrenal cortex, a process which occurs after sexual maturity in females and earlier in males, was described by one of us in 1927 (11). It was then suggested that the mouse X zone might be comparable to the human fetal reticularis, primarily because its degeneration constituted a spontaneous regression of a large part of the inner portion of the cortex, occurring apparently as a part of the normal maturation of the gland. The two juxtamedullary zones are somewhat similar in appearance in that they are composed of cells in a reticular arrangement, with a fine granular cytoplasm in contrast to the spongy vacuolated cytoplasm of the cells in the columns of the outer zona fasciculata. The mouse X zone was found not to stain in Sudan III in its intact state in contrast to the permanent cortex which stains very strongly. The X zone became Sudan positive during its involution (11). Recently Jones (17) has made a histochemical study of the X zone, finding that not only is it Sudan IV negative, but it is also Schiff negative and contains no birefringent material.[2]

[1] Present address, Mayo Foundation, Rochester, Minn.

[2] Jones, in Endocrin. *45*, 514, 1949, presents further studies on the mouse adrenal, using the Schiff reagent, in a paper entitled "The Relationship of the Mouse Adrenal Cortex to the Pituitary."

The human adrenal cortex, at the seventh month of gestation, has been found to stain lightly with Scharlach and Sudan III throughout, but more strongly in the outer rim, while at full term fat was present only in the outer rim and not in the main body of the fetal cortex (7, 18). As far as we are aware, the human fetal adrenal has not been studied by more specific histochemical methods.

However, these features of similarity contain nothing specific, and in the histogenesis of the two zones there are obvious dissimilarities. The histogenesis of the human adrenal has recently been carefully studied by Gruenwald (9), who concludes that the adrenal cortex develops by rapid differentiation of mesenchyme of the coelomic wall during the first phase and a slow continuation during a second phase rather than a succession of two separate proliferations, as suggested by Keene and Hewer (18) and Uotila (23). Furthermore, "it is impossible at any stage of intrauterine life to draw a sharp line between cells which differentiated at different times in the early embryo. . . . It is, therefore, unjustified to speak of separate anlagen of the X zone (i.e. the fetal reticularis) and the permanent cortex" (9). The histogenesis of the X zone of the mouse has recently been studied by McPhail and Read (19) who substantiate our previous views (11, 12) that no conclusive evidence could be found to support the idea that the X zone is a transitory separate development from the cortical anlage, but that it is probably derived from the fasciculata.

The development of a new histochemical technique for obtaining a color reaction with lipoidal carbonyl groups presents an opportunity for a new approach to the question of the homology of the mouse X zone and the human fetal reticularis. The histochemical application of a hydrazine reaction to the adrenal was first made by Bennett (3). A substantially improved procedure has recently been introduced by Ashbel and Seligman (1). In this method, formalin fixed tissue is treated with a hydrazine derivative, which reacts with active carbonyl groups to form nearly colorless compounds, which are converted by coupling with a tetrazonium compound into an intensely blue azo dye. Ashbel and Seligman reported that adrenals of rats, rabbits, dogs, and humans react in this way, as well as granulosa cells of the ovary, luteal cells, interstitial cells of the testis, and syncitial cells of the placenta, whereas a number of other tissues tested gave no color. Nerve myelin sheaths give a blue color which differs from that in the

adrenal and gonads in that the reacting material is not soluble in acetone.

This procedure, we have found, indicates a clear cut differentiation between mouse and human adrenals, in that the human fetal reticularis is strongly positive for the presence of lipoidal carbonyl groups, whereas the mouse X zone gives no indication of the presence of significant amounts of carbonyl groups. The details of the study will now be presented.

METHODS

Mice of the H strain bred in this laboratory, and three mice born of an H strain female and a wild male were used for this experiment. They were killed by decapitation and in all but two mice the adrenals were removed at once and placed ln 10% phosphate buffered formalin at pH 7.4 for several days to several weeks. In order to test the effect of delayed fixation, two mice were killed, allowed to remain at room temperature for four hours and in the icebox 18 hours before the adrenals were removed and fixed. There was no appreciable change in the carbonyl reaction after delayed fixation. Males were castrated through an abdominal incision under ether anesthesia, at 20 to 22 days of age. Human adrenals were obtained at routine autopsies, 2 to 25 hours following death in most cases, and were fixed in buffered formalin. Causes of death are listed in the table.

Frozen sections 20 to 30 micra thick were stained according to the procedure of Ashbel and Seligman (1) with minor modifications. The frozen sections were washed 3 to 4 hours in several changes of water. The ketone groups were allowed to react with the hydrazide by soaking the sections 1 to 2 hours in the solution of 2-hydroxy-3-naphthoic acid hydrazide, prepared according to the method of Seligman, Friedman and Herz (22), then washed 2 hours in three to four changes of 50 per cent aldehyde free alcohol prepared according to the method of Danner and Hildebrand (6). They were then transferred to 25 per cent alcohol to lessen the violence of mixing currents, and then washed 2 hours in two to three changes of distilled water. The reaction product was then coupled with the stabilized tetrazonium compound (Dupont Naphthanil Diazo Blue B[1]) in a solution of half phosphate buffer at pH 7.4 and half 95 per cent aldehyde free alcohol. This treatment causes a blue color to appear very rapidly in sections floating in a yellow to brown reaction mixture. After a final washing, sections were mounted in Kaiser's glycerogel.

Sections were also cut from paraffin embedded tissues. In most human cases these were from the same block of tissue as was used in making the frozen sections. In the case of the mice, serial sections of the opposite adrenal were made. The paraffin sections were 10 micra thick, and were stained in Ehrlich's acid hematoxylin and eosin.

[1] Dupont Naphthanil Diazo Blue B contains 20% tetrazotized diorthoanisidine, 5% zinc chloride, and 20% aluminum sulphate.

There has been some discussion in the literature as to the specificity of tests based on hydrazine reactions. These considerations are well summarized by Ashbel and Seligman (1) and it is here assumed that in the formalin fixed adrenal a blue color is indicative of ketosteroids. Unfortunately, one can draw no conclusions as to the type of adrenal steroid present.

Some variation was observed in the quality and intensity of the color reaction in sections of the same adrenal stained at different times, so that we are not able at present to use the reaction to make quantitative comparisons as to the steroid content of the glands of different individuals under different circumstances. Without magnification the color of the sections varied from a light greyish-blue to a dark blue-black. Microscopically, a clear sky blue was obtained after washing the stained section in acetone for one hour. Without this treatment the mouse adrenals usually showed a darker purple-blue, which sometimes appeared in human sections, but often the human adrenals appeared more grey-blue.

Tissues reported not to stain by Ashbel and Seligman, such as adipose tissue and kidney, sometimes showed a very pale lavender color microscopically, when carried through the solutions at the same time as an adrenal giving a strong color. Although the colored compound is relatively insoluble, it seemed possible that some disintegration of the adrenal tissue might release colored material in suspension or solution which then might be non-specifically absorbed by non-reacting tissue. Consequently adrenal areas showing a pale lavender color were not classified as positive. However, in most cases, and unless otherwise recorded, non-reacting tissue showed only a yellow tinge. Cortical sinusoids contained colored material, as noted by Ashbel and Seligman.

RESULTS AND OBSERVATIONS

Mice adrenals. In a total of twenty-three mice, the outer part of the zona fasciculata always showed the strong blue color characteristic of the carbonyl reaction (See photographs of sections). The glomerulosa was more variable. If color was present in the glomerulosa at all, it was usually less intense than in the fasciculata. The glomerulosa was often different in various regions of the same gland, some showing a blue color and others not. In eight of the twenty-three mice, the glomerulosa showed no blue color. The color of the glomerulosa did not appear to be related to age or sex. The medulla was uniformly unstained. Two adult parous females and two intact adult males were without X zone, as expected, while five virgin females, 21 to 70 days of age, and fourteen castrated males, 36 to 70 days of age, showed X zones which were somewhat variable in size, being largest in the castrated wild mice stock, where over half of the width of the cortex was X zone. Whether or not the X zone was present, the blue color of the carbonyl reaction was less intense or absent in the inner part of the fasciculata, and in

the narrow region which may be considered the permanent reticularis in the mouse.

The X zone was without any blue color in every case except in two mice, where it took a pale lavender color, which was not considered positive for the reasons discussed above. Where vacuolation of the X zone had occurred, showing the beginning of involution, the vacuoles were also unstained.

In most sections carried through the carbonyl test scattered cells were seen with a brilliant red color in the cytoplasm. These cells were most conspicuous in the X zone, most frequently near the medullary border, but were occasionally found in the inner fasciculata. These crimson cells were slightly larger than the surrounding cells of the X zone, and contained large round unstained nuclei. They could not be identified as a distinct type of cell in the hematoxylin and eosin stain. They appeared to be an integral part of the adrenal parenchyma. A red color is thought to result from coupling of the hydrazide to protein through the naphtholic group, so that the tetrazonium compound reacts with the free hydrazino group of the hydrazide (1).

Human adrenals. The series of newborn infants studied by means of the carbonyl reaction are described in the table. In these seven adrenals it was found that while the glomerulosa in some instances showed less color, the remainder of the cortex either stained uniformly or else stained more intensely in the central region. There was no tendency for the fetal reticularis to stain less intensely than the outer or permanent cortex. On the contrary, although the series is too small to make the conclusion binding, there seems to be a tendency for the color of the fetal reticularis to become more intense in the larger infants. In the two largest there was a clear cut transition from an outer, moderately colored area, to a central intensely colored area, which occupied roughly the inner half of the cortex. Many questions arise as to the cause of this demarcation. One of these glands is illustrated in the accompanying photographs.

Sections of this gland soaked in acteone overnight before treatment with the hydrazide did not develop the blue color, indicating that the reacting material is slowly soluble in acetone.

In addition to the series of newborns, three other cases were examined by means of the carbonyl reaction. One, a child of ten months,

with a diagnosis of congenital stenosis of the pulmonary artery, had adrenals in which the fetal reticularis was entirely absent. The remaining cortex stained fairly uniformly except for a narrow unstained band between the glomerulosa and fasciculata. We were unable to obtain a significant degree of color in the adrenals of a child of 11 years, who died 3 days after severe burns. A fairly uniform blue color was observed in the adrenals of a woman aged 62 with cirrhosis of the liver, who died following severe gastro-intestinal hemorrhage.

TABLE 1

The Carbonyl Reaction in the Adrenals of Human Infants

CASE NUMBER	SEX	BODY WEIGHT	POSTNATAL AGE	CAUSE OF DEATH	CARBONYL REACTION
		grams	*days*		
031	F	725	0	Prematurity, no respiration	U
044	F	1770	0	Unborn. Fatal maternal poliomyelitis	U
Cl	M	1405	0	Agenesis of kidneys, no respiration	U
108	M	2245	0	Hydrocephalic, no respiration	G
050	M	1910	1	Atelectasis	G
051	M	2830	1	Atelectasis	S
019	M	2460	6	Meningeal tears, rt. lung consol.	S

U, uniform color intensity except for localized variability, or less reaction in the glomerulosa, but otherwise no zonation or gradual progression.

G, Gradual increase in color intensity to a maximum in the central part of the cortex.

S, sharp transition from moderate reaction in the outer half of the cortex to a more intense reaction in the inner half.

The case numbers are the last three digits of the Johns Hopkins Hospital autopsy numbers, from cases during the autumn, 1949, except for Cl, which was obtained from the Carnegie Institute, Department of Embryology.

In addition to the adrenals studied by means of the carbonyl reaction, we have examined the adrenals of twenty other infants from birth to 6 months of age stained by routine hematoxylin and eosin. In agreement with Elliott and Armour (7) and Gruenwald (9) we do not observe any clear separation of the newborn adrenal into the so-called fetal zone and a permanent cortex. There seems to be no visible demarcation prior to the beginning degeneration of the inner area.

In early stages there is a peripheral layer of small non-eosinophilic cells, which would generally be considered as glomerulosa, while the

main mass of the gland is composed of larger eosinophilic cells. When degeneration takes place, however, it does not extend up to the glomerulosa. The degenerating cords seem to be continuous, in early stages, with cords of cells which are not degenerating. Our observations in this respect are in agreement with Gruenwald (9) who states: "the outer layer of the embryonic cortex has been considered either as the zona glomerulosa or as the permanent cortex, in which case it would correspond to both the zona glomerulosa and the permanent zona fasciculata of the postnatal gland . . . the existence of fluid transitions between zones makes any comparison of layers at different stages very unreliable." After the postnatal degeneration "some of the large cells always remain intact and eventually become part of the permanent zona fasciculata. . . . The greater part of the inner layer of large cells degenerates during the first few months of extra-uterine life. Its demarcation from the permanent cortex is not a sharp one until the process is far advanced" (9). It would seem that the degenerative process is more probably determined by functional factors than by cell lineage.

DISCUSSION

The fact that lipoidal carbonyl groups in the adrenal do not react, according to Ashbel and Seligman (1), unless they have been previously unmasked by formaldehyde treatment, makes it impossible at present to exclude the possibility that the X zone contains ketosteroids which remain unmasked. Allowing the adrenals to autolyze at room temperature for four hours before fixation produced no appreciable change in their reactivity to the carbonyl reagent. In any case, since the X zone shows no evidence of reactive carbonyl groups, it appears probable that its activities are different from those of the fasciculata.

Bennett (3) provisionally considered the part of the zona reticularis which does not show evidence of free ketosteroids, in the cat, as a post-secretory zone, regarding its component cells as having passed the secretory stage in their life cycle. However, as Bennett pointed out, the possibility remains that the steroid molecules might be coupled with some other molecule, such as a sugar or ascorbic acid (26) before secretion takes place. "The post-secretory zone might well be involved in the secretion of such as yet hypothetical water soluble sterone

glycosides as Zwemer and Lowenstein (26) postulate, in which case the present term applied to it would be inappropriate" (3). The facts that the mouse X zone is without free ketosteroid, and that it occurs as an independently proliferating part of the gland (Whitehead, 25) suggest that such cells may have a special function. The X zone resembles the reticularis under some conditions, not yet completely defined, in which the reticularis cells are without free ketosteroid. This situation redirects attention to the possibility that the reticularis cells without free ketosteroid may have a special function, and are not merely waiting for sensescence under all circumstances. If ketosteroid is conjugated to a water soluble molecule it might be dissolved out during processing of the sections.

The finding that the X zone of the mouse and the fetal reticularis of the human are completely different in their content of free carbonyl groups certainly indicates that there can be no simple homology between them, and substantiates histogenetic indications of difference. Although our observations are quite in agreement with those of Gruenwald (9), insofar as they are pertinent, we question whether in the light of present findings, the term *X zone* should be applied to the human fetal reticularis. The term *X zone* was introduced (11) to describe a specialized zone in the mouse adrenal which is characterized in several rather definite ways. The part of the adrenal which degenerates postnatally in the human does not represent the entire fetal adrenal; therefore we have here preferred to use the term *fetal reticularis* rather than *fetal zone*. In this respect we follow the usage of Blackman (4) for the human adrenal. On the other hand, we feel that the term *X zone* has a specific justification in the case of the mouse, until it can be replaced by a functional description.

The X zone of the mouse is specifically inhibited by androgens (13, 17) but it does not appear to be the source of physiologically appreciable amounts of androgens, since adrenalectomized castrated mice, maintained in good condition on a synthetic high sodium low potassium diet, do not show any greater impairment in the prostate and seminal vesicles than do litter mate castrates having intact adrenals with fully developed X zones (15). In the young rat, on the other hand, this procedure demonstrates that the adrenals do excrete physiologically appreciable amounts of androgens for a brief period in the course of

their development (14). A further characterization of the X zone consists in a relationship to the level of dietary protein. It is almost completely suppressed by a low protein diet, is well developed on a diet moderately high in crude casein, and is appreciably reduced at extremely high casein levels (16). However, there is no reasonably satisfactory concept of its role.[1]

There are several good reasons for a re-examination of the functional significance of the fetal reticularis and its degeneration. First of all, the considerations that impelled Grollman (8) to apply the term *androgenic zone* to the mouse X zone and the human fetal reticularis have been shown to be without foundation as far as the mouse X zone is concerned (15), while Carnes (5) found no evidence of androgens in the human fetal cortex. Finally, we now find that the supposed homology between the two adrenal areas is not supported by a relatively specific histochemical test. The postnatal regression of the human adrenal does find a parallel in the reduction in the size of the human uterus after birth (20, 21). The possibility that the adrenal regression is a similar result of the withdrawal of certain maternal hormones at birth merits consideration. The urinary excretion of glucocorticoids has been found to be low in the newborn, rising during the period of reticularis involution (24), when the cells of the fasciculata are becoming organized into their typical columns.

SUMMARY

1. The X zone of the mouse adrenal shows no evidence of the presence of lipoidal carbonyl groups, in contrast to the zona fasciculata which is strongly positive, and to the zonae glomerulosa and reticularis which are sometimes positive but generally stain less intensely than the fasciculata.

2. The zona reticularis of the human adrenal at birth is strongly positive for lipoidal carbonyl groups. This is evidence against any simple homology between the mouse X zone and the human fetal zona reticularis.

[1] The X zone is further differentiated from the fasciculata in that it is not maintained by ACTH, but is dependent on a pituitary LH, according to Jones, Endocrin. *45*, 514, 1949.

ACKNOWLEDGMENTS

We are greatly indebted to George W. Corner and to William H. Carnes for their courtesy in providing us with material, and to Kenneth C. Blanchard and Leslie Hellerman for their advice regarding the preparation of reagents. Dupont Naphthanil Diazo Blue B was kindly donated by the Dupont de Nemours Corp., Wilmington, Del.

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PLATE I

Upper: Adrenals of normal adult male mouse. × 150

FIG. 1. The reaction for lipoidal carbonyl groups is evident as the dark tone in the glomerulosa and fasciculata, and is absent in the inner part of the cortex. The medulla, also non-reacting, occupies the lower third of the section. Sinusoids in the fasciculata, reticularis, and medulla also contain stained material. The nuclei are unstained.

FIG. 2. The opposite adrenal of the same mouse, hematoxylin and eosin stain. There is no sharp line of transition between the fasciculata and reticularis, but the cells in the region of the reticularis tend to have a fine granular cytoplasm in contrast to the more spongy cytoplasm of the fasciculata.

Lower: Adrenals of prepuberally castrated mouse. × 150

FIG. 3. The reaction for lipoidal carbonyl groups is present in the fasciculata and in part of the glomerulosa. The inner part of the fasciculata and the X zone are negative, as is the medulla, which occupies the lower left area.

FIG. 4. The opposite adrenal of the same mouse, hematoxylin and eosin stain. This shows a sharp transition between the fasciculata and the X zone. Medulla occupies approximately the lower quarter of the section.

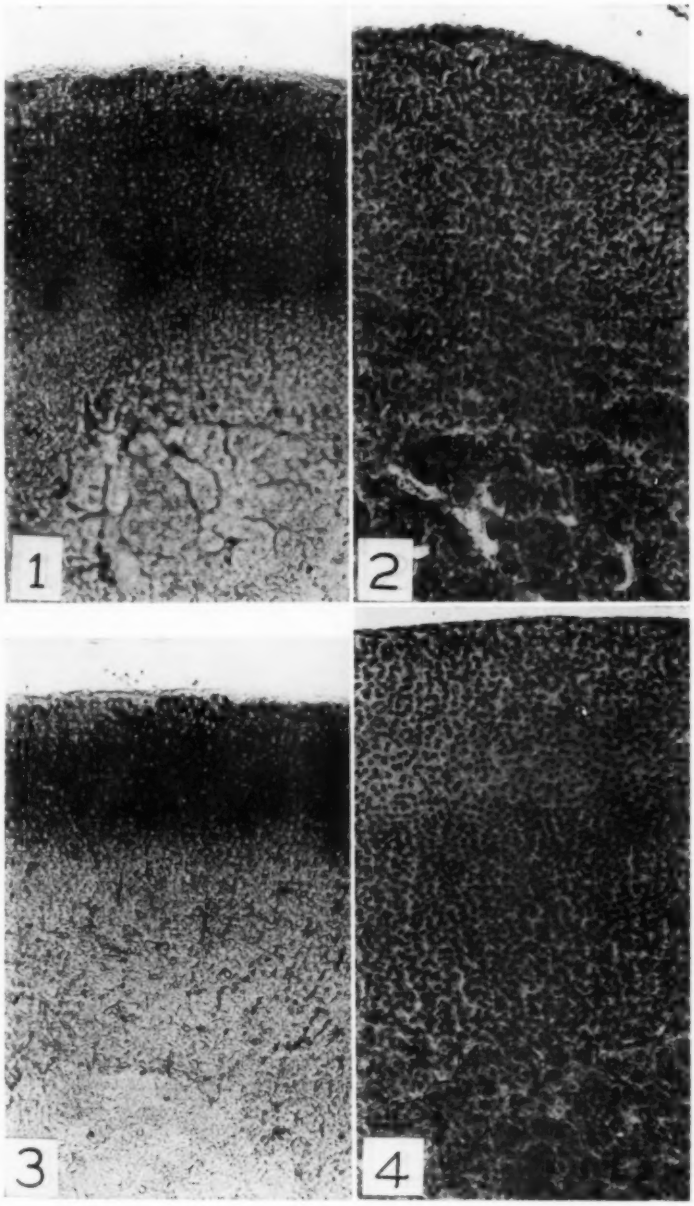

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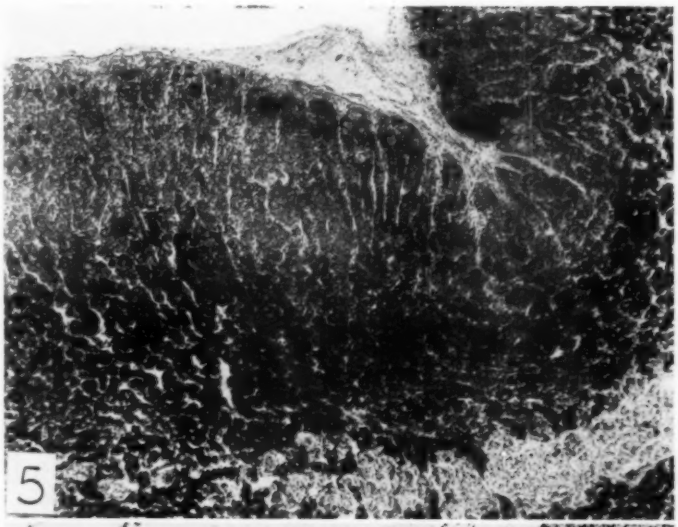
PLATE II

Adrenal of a human infant (Case 019) × 60

FIG. 5. The reaction for lipoidal carbonyl groups is strongest in the central reticularis, and is definitely positive in the glomerulosa and fasciculata, which may be contrasted with the non-reacting strip of medulla across the lower right corner.

FIG. 6. Hematoxylin and eosin stain of a section from the region shown above. Fasciculation of the outer part of the cortex is under way. There are large vacuoles in the cytoplasm of the zona reticularis cells which presumably correspond to the non-reacting vacuoles in the upper photograph. The clumps of medullary cells are intensely stained with hematoxylin.



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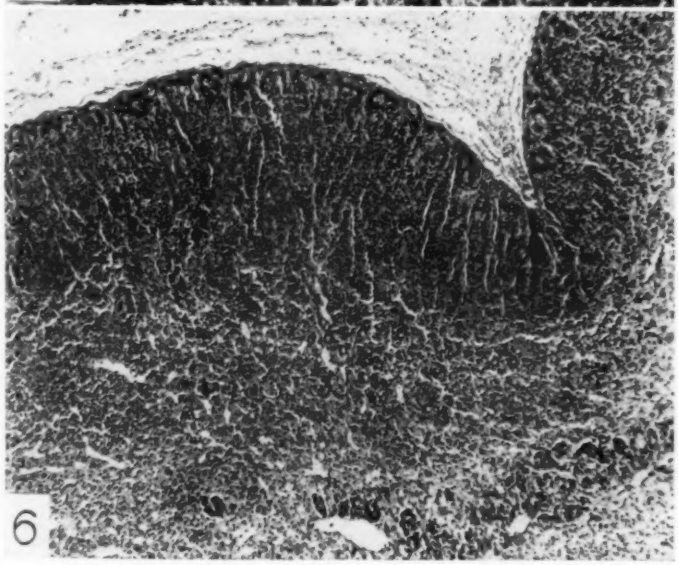

6

THE NATURAL COURSE OF A LIGHT EXPERIMENTAL INFECTION OF SCHISTOSOMIASIS JAPONICA IN MONKEYS*

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Received for publication January 24, 1950

INTRODUCTION

Schistosomiasis japonica is a common endemic disease in the Orient, notably in Japan, China and the Philippine Islands. With the invasion of Leyte, Philippine Islands, in October 1944, when some 1500 U. S. soldiers (1) were infected, it became a significant military problem. Although numerous clinical and experimental studies of Schistosomiasis japonica have been reported during the past few years, none has attempted to deal with light infection in monkeys. Such infections might be expected to simulate more closely the condition in veterans than other experimental infections. For this reason we have had under continual observation for the past four and a half years some fifteen experimentally infected *Macacus philippinensis* monkeys. All of those still under observation maintain an active infection, but remain in apparent good health despite the lack of treatment.

MATERIALS AND METHODS

Of the fifteen *M. philippinensis* monkeys infected with *S. japonicum*, twelve were infected with an estimated 100 motile cercariae from crushed snails obtained from a naturally infected area on Leyte, P. I. This was done in the course of experimental chemotherapy studies reported elsewhere (2). One group of five was infected in April 1945, the other seven in July and September 1945.

Three other monkeys were infected in November of the same year with an estimated 200 motile cercariae during continued studies in Kofu, Japan and thus represent another source of *S. japonicum* (3). In October 1947, two years later, when a study of the immune mechanisms in schistosomiasis was undertaken, two of the monkeys originally infected in the Philippine Islands with *S. japonicum*, were then given 1000 *S. mansoni* cercariae. All cercariae were injected intraperitoneally. These two are the only doubly infected monkeys in this study. The

* Supported by a contract with the Veteran's Administration.

M. philippinensis were obtained from the natives by barter and varied from an estimated one to three years of age.

Three monkeys were killed and autopsied 2½, 3 and 6½ months after date of infection respectively. One became moribund and was bled to death two years after infection. One died about three years after infection. Another two were accidentally killed four years after infection. A complete autopsy was performed on two of the four that died.

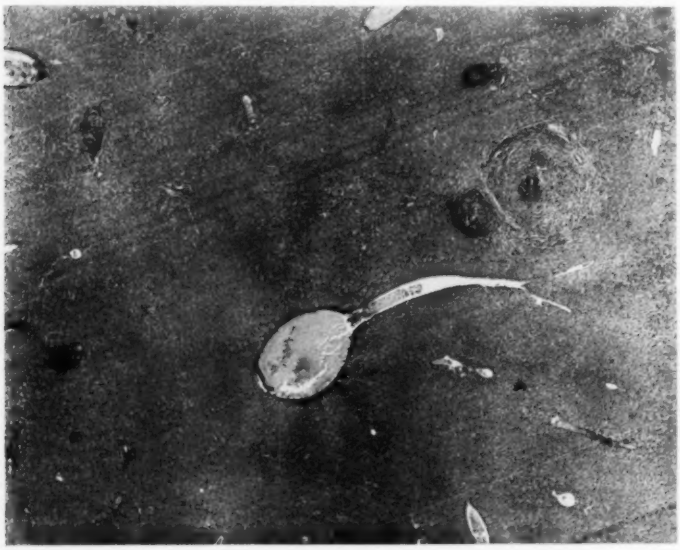


FIG. 1. Liver from monkey 2½ months after *S. japonicum* infection. There are many scattered pseudo-tubercles containing living eggs but no cirrhosis. ×25

All of the monkeys infected with *S. japonicum* had been brought back to the United States a few months after infection. They were kept in cages at the Rockefeller Institute, Princeton, New Jersey for the first year. Since then they have been kept in a large room with an outdoor runway at the Johns Hopkins Medical School. They are placed in separate cages when stool egg count studies are undertaken. The cages are so constructed that the stools fall into a removable pan in which a 24 hour stool specimen can be collected daily. The routine food for our experimental monkeys consists of dog biscuits supplemented by assorted vegetables and fruits, particularly, fresh sweet potatoes, turnips, apples and oranges. The fresh fruit is given approximately twice a week, and the food is withheld entirely one day a week. Experimental changes in diet will be discussed later.

About four years after the infection, a female monkey went through a successful pregnancy and gave birth to a male baby monkey.

METHODS FOR STOOL EXAMINATIONS

Water sedimentation: A sample of stool was emulsified in about 250 ml. of water. This was poured into a conical urine glass through three layers of rough gauze. The glass was filled with tap water, the supernatant poured off after stand-

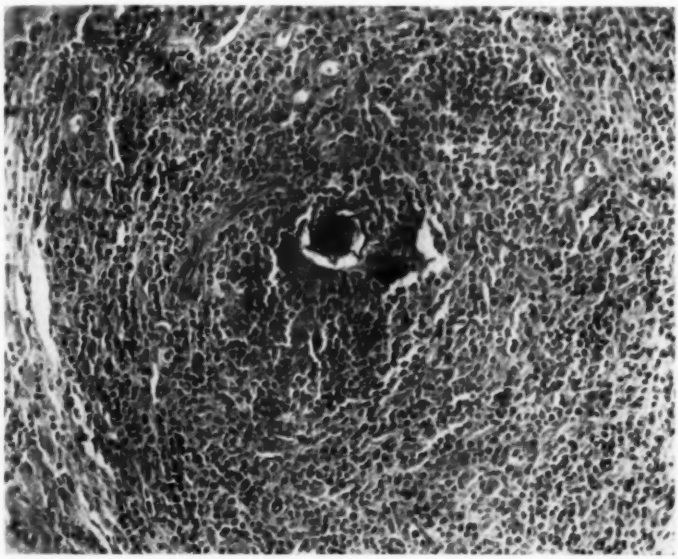


FIG. 2. Same as in Fig. 1 under higher magnification to show a single pseudotubercle. ×200

ing for about a half hour, and the glass refilled with tap water. This procedure was repeated until the supernatant was clear and a portion of the sediment was then examined under a large cover slip (22x44 mm.). The stool was not called negative unless all of the sediment present under this cover slip had been examined (3).

Quantitative Acid-Ether-Xylol: This method is a modification of the Telemann technique by Loughlin and Stoll in 1946 (4). In this modified technique 4 grams of feces was suspended in 56 ml. of water; then 1.5 ml. of this fecal suspension was transferred to a centrifuge tube and added 3.5 ml. 20% hydrochloric acid and 5 ml. of freshly prepared mixture of equal parts ether and xylol. A determination of all the eggs in the sediment gives the number in .1 gram of feces.

In our studies we used the entire specimen in order to get a more adequate sam-

pling. We suspended the whole weighed 24 hour specimen in water to make a 1 to 15 dilution. $\frac{1}{10}$ of this suspension was taken out and 20% hydrochloric acid and ether-xylol mixture were added in calculated proportions. The egg count obtained from the sediment was the number of eggs in $\frac{1}{10}$ of the total 24 hour stool specimen instead of 0.1 gram described by Loughlin and Stoll. We believe that suspension of the whole specimen gives more consistent results in monkeys, because the schistosome eggs are quite irregularly distributed; also, it is more practicable when dealing with the smaller stool specimen.

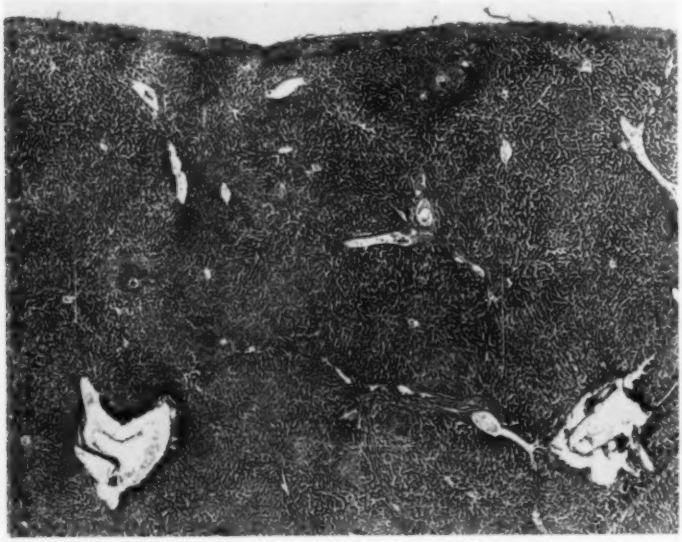


FIG. 3. Liver from monkey 4 years after *S. japonicum* infection. There are pseudotubercles but no appreciable cirrhosis. ×25

Alcohol sedimentation: A 24 hour stool specimen was emulsified in about 250 ml. of 10% ethyl alcohol in a beaker. This was poured into a sedimentation cone after being passed through a fine metal strainer. After standing for about an hour the supernatant was poured off gently and the cone refilled with 250 ml. of 10% alcohol. The supernatant was decanted again after about half an hour and the process repeated twice or more until the supernatant was clear. 0.05 ml. of the sediment was sucked up with a 1 ml. pipette at three different levels, upper, middle and bottom. Each was transferred to a glass slide, covered with 22x40 mm. cover slip, and the whole smear examined under the microscope with low and high dry objectives (5).

INCUBATION PERIOD

The exact incubation period in human cases is difficult to calculate, because the dates of initial exposures are usually not clearly remembered or known. This is particularly true within an endemic area. Thus, estimates of the time between exposure and the demonstration of eggs n the stool has varied from 26 days to 6½ weeks (6, 7, 8). With the

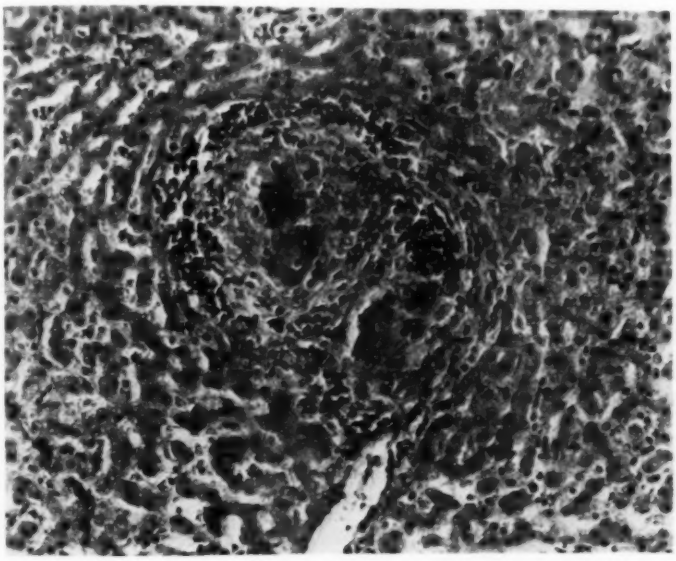


FIG. 4. Same as in Fig. 3 under higher magnification. ×200

sudden outbreak of the disease in American soldiers on Leyte, more exact and complete data became available. An analysis of these data (9) indicates that the latent period in cases eventually presenting the more severe symptoms was usually, but not always, shorter than that in cases with mild symptoms. In 45 patients presenting severe or moderately severe symptoms, the shortest incubation period was 28 days, the longest 60 days with an average of 41 days. In patients with mild symptoms, the shortest was 26 days, the longest 79 days, the average 50 days. In 14 patients, eggs were demonstrated in the stools 7 15 days after the onset of symptoms.

In experimental animals, Cram found that a monkey infected subcutaneously with 300 *S. japonicum* cercariae and dogs given 300–500 cercariae partly by subcutaneous injection and partly by spraying into the cheek cavity showed incubation periods of 40–42 days (10). In guinea pigs it has been found that the worms matured in 4–5 weeks,

TABLE I

The appearance of ova of Schistosoma japonicum in the stools of experimentally infected animals

MONKEYS	NUMBER OF DAYS AFTER INFECTION																							WORMS OBTAINED AT POSTMORTEM
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
#7	0	0	0	0	0	0	0		0	0	0		0		I	ML	ML D	ML							
#8	0	0	0	0	0	0	0	0	0	0	0		0	0	ML	ML	0	ML MD							
#9	0	0	0	0	0	0	0	0	0		0		0		0	ML	0	ML							
#10	0	0	0	0	0	0	0	0	0		0		0		I			ML							
#11	0	0		0	0	0	0	0	0	0	0		0	0	ML	ML	ML	MD							
																	ML MD D	ML MD							
Dogs																	I								
#1*						0	0		0		0	0	D	MD			ML D								36
													ML												
#2*							0	0	0	0	0	0	MD D												38
#3										0	0	0	0	0	0		0	MD	0	ML	ML MD				
#4								0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	MD	4

* Puppies (inoculated by placing cercariae on skin)
I—Immature Eggs
ML—Living Mature Eggs
MD—Dead Mature Eggs
D—Degenerate Eggs

so that egg deposition in the submucosa of the intestine and in the liver is uniformly present in 5 weeks (2).

In this study, stool examinations were done on all of the monkeys, either prior to injection of cercariae or during the early incubation period, to rule out pre-existing schistosomiasis infection. Five monkeys infected on July 30, 1945, in Leyte were subjected to daily stool examinations by water sedimentation technique starting on the 22nd day after the infection. Table I gives the results of the stool examination of these animals and also includes observations on the incubation period in four experimentally infected dogs. It was observed that the appear-

ance of typical mature eggs in all cases occurred either in the first or the second positive stool. The incubation period was 36–37 days in these light infections of the monkeys. The incubation period in the dogs was 34–35 days. These observations show that typical mature forms of the eggs are present early in acute mild infections of Schistosomiasis japonica. The appearance of typical mature eggs in all cases by the second

FIG. 5. Liver from monkey 3 years after *S. japonicum* infection. There are pseudotubercles with minimal early cirrhosis. ×25

day of positive stools seems to indicate that in few, if any mild cases, need the diagnosis rest on other than the mature type of egg. One of the dogs autopsied was harboring only 2 pairs of worms. This result also indicates that the water sedimentation technique is adequate for the detection of eggs even in minimal infections, but only during the acute phase of the disease.

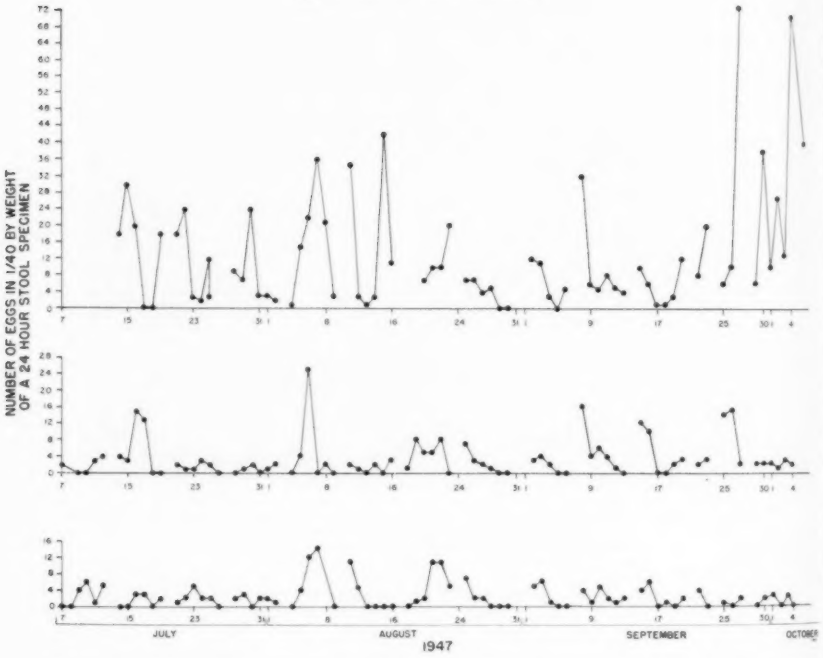
At the end of the incubation period, the monkeys were listless and had a mild diarrhea containing blood and mucus. No attempts were made to have complete blood, urine or other clinical studies done.

VARIATIONS OF EGG OUTPUT

A. On Routine Diet

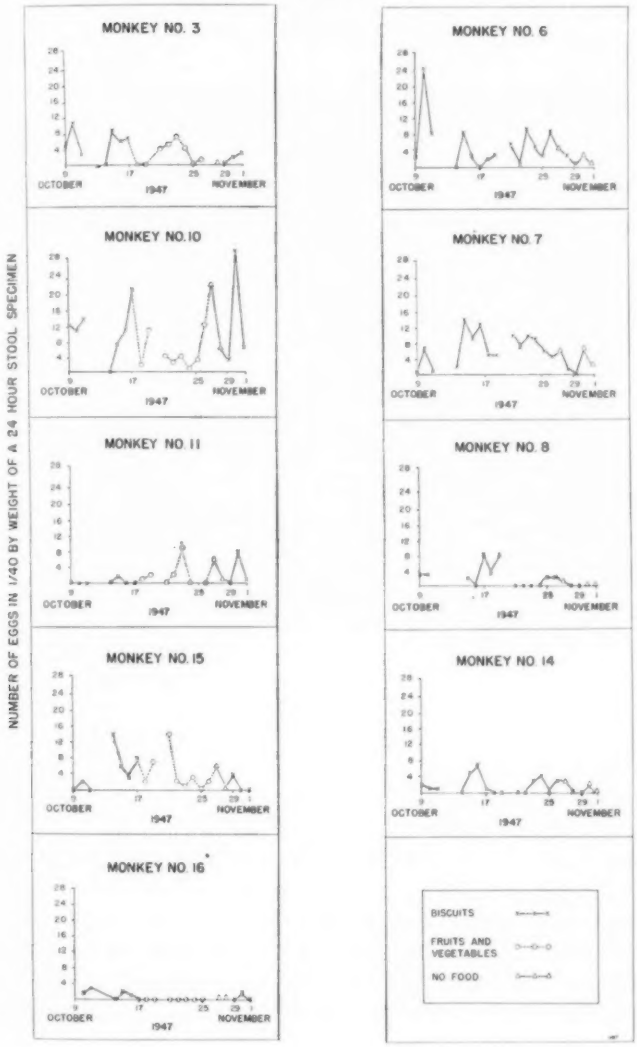
The original purpose of this study was to determine the average duration of infection with *S. japonicum*. Infections with *S. mansoni*

CHART I.
Daily Stool Egg Count
Monkeys on Routine Feeding
(Quantitative AEX Method)



have been reported lasting as long as 26 years (11). At two years, repeated examinations of the stools by direct smears and water sedimentation showed that it was not always easy to demonstrate such infection. For this reason, daily stool examinations were made with the Quantitative Acid-Ether-Xylol concentration method. All animals were found to be infected. Chart I shows the variable number of eggs found on the daily stool examinations of three monkeys between

CHART II.
Daily Stool Egg Count
Monkeys on Changing Diets
(Quantitative AEX Method)



July and October 1947. Throughout the period of these examinations, our monkeys were on the feeding routine previously described.

There seems to be no regular pattern and the eggs seem to appear in showers. There is a very high output on some days and a negative result with AEX technique on other days.

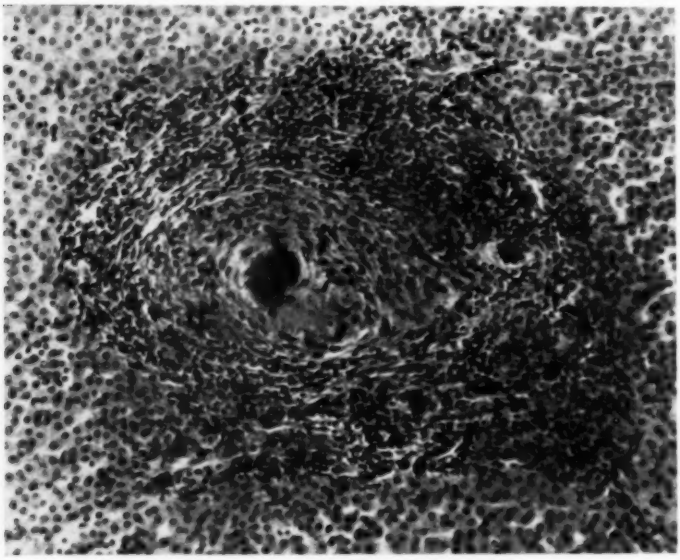


FIG. 6. Same as Fig. 5 under higher magnification. ×200

B. On Changing Diets

Since the diet changed markedly from day to day we decided to see if such abrupt changes had any effect on the egg output. To do this we studied nine monkeys for 23 days. They were divided into two groups, five in the first group and four in the second. The first group of monkeys were fed with biscuits and water for nine days. From the tenth day on, the first group of monkeys were fed with fruits and vegetables for a period of eight days followed by no food for two days and then back on biscuits again. The second group of monkeys were fed with biscuits every day, except the last three days on which they received no food. Daily stool examinations, using the same AEX tech-

nique, were done on all of them. The results are shown on Chart II. The egg output was not influenced apparently by the change of diet.

C. Comparison of AEX and Alcohol Sedimentation Methods on Stool Examinations

Between September 1948 and January 1949, about three years after infection, a series of stool examinations by AEX method were done on

TABLE II*

10% Alcohol Sedimentation

(Eggs found in 0.05 ml. of sediment)

DATE	MONKEY #3			MONKEY #15		
	Upper Layer	Middle Layer	Bottom Layer	Upper Layer	Middle Layer	Bottom Layer
6/13/49	0	0	0	2 I	0	0
6/16/49	1 M	0	1 I	1 D	0	0
6/18/49	1 D	1 I		No Specimen		
6/24/49	0	0	2 D	0	0	0
6/28/49	2 ML	3ML, 2I, 2D	1 I	1 ML	0	1 ML
6/30/49	0	2 ML	1 I	0	0	0
7/ 5/49	0	0	0	No Specimen		
7/ 7/49	1 MD	0	0	0	0	0
7/11/49	0	0	3 ML	2 ML	1 MD	1 D
7/13/49	0	2 ML	1 ML	2ML, 1 I	1 ML	0

* Quantitative AEX stool examinations were done on alternate days. In monkey #3 only one positive stool was obtained out of twelve examinations and on another occasion there was a fleck of bloody mucus containing rectal mucosa and 15 eggs by direct smear, but AEX was negative. In monkey #15, also only one positive stool was obtained out of eleven AEX examinations.

ML—Living matured eggs
I—Immature eggs
MD—Dead mature eggs
D—Degenerate eggs

two monkeys. Near the end of this period, the egg output of the monkeys was decreasing and by the AEX method we could not obtain a positive result as easily as in the beginning of this serial stool examinations.

In June and July 1949, four years after infection, another series of daily egg counts was performed. In addition to the quantitative AEX method, we added the 10% alcohol sedimentation method to compare the effectiveness of these two. In one monkey, one positive stool was

obtained out of twelve AEX examinations, while there were eight positive stools out of ten examinations by alcohol sedimentation. In the other monkey there was also one positive stool out of eleven AEX examinations, but there were five positive out of eight alcohol sedimentation examinations. Results are shown in Table II.

It is apparent that the 10% alcohol sedimentation method is more efficient than the AEX in detecting schistosome eggs in a stool specimen, although the alcohol sedimentation is not a quantitative method. This may be for two reasons. The alcohol sedimentation technique allows one to concentrate the entire stool and examine portions of the concentrate. Secondly, the miracidia remain alive with this procedure and subsequent hatching is possible. We also found that the upper and middle of the sediment layers have more schistosome eggs than the bottom layer. This is probably because the heavier particles settled down first during the process of sedimentation and the eggs rest on top of them.

PATHOLOGICAL STUDIES

Three monkeys were killed and autopsied during the acute stage of infection, 2½, 3 and 6½ months from date of infection respectively. In all of them, well developed freely movable adult worms were found in the mesenteric veins, chiefly those leading from the large intestines. Thirteen pairs and one male were found in one monkey; five pairs in another and nine pairs in the third monkey. There was no ascitic fluid. The livers were not appreciably enlarged. A few yellow flecks and nodules were found on the surface of the liver. Two female worms were found in one liver and one pair of male and female worms were found in the other one. The spleens appeared to be normal. The large intestines showed no apparent ulcerations, but small reddish papules were seen on the mucosa. In one monkey the lungs showed numerous grayish flecks and nodules of pinhead size or smaller on the surface. These nodules contained schistosome eggs when crushed preparations were examined under the microscope.

Microscopic examination: Numerous eggs were found in the liver and intestines, and as mentioned above, in the lungs of one monkey. There was no cirrhotic change in the liver. In the parenchyma of the liver, the eggs were usually located near the portal system. A foreign

body reaction sometimes with giant cells was usually seen around the egg shell with a pseudo-tubercle formation. Around the egg and the miracidium, there were mononuclear cells and polymorphonuclear leucocytes, many of them were eosinophiles. A kind of brown hematin pigment, very similar to the malaria pigment, was also found in the Küpffer cells. In the intestine, the eggs were usually found under the mucosal area with similar changes. In one section organized thrombi

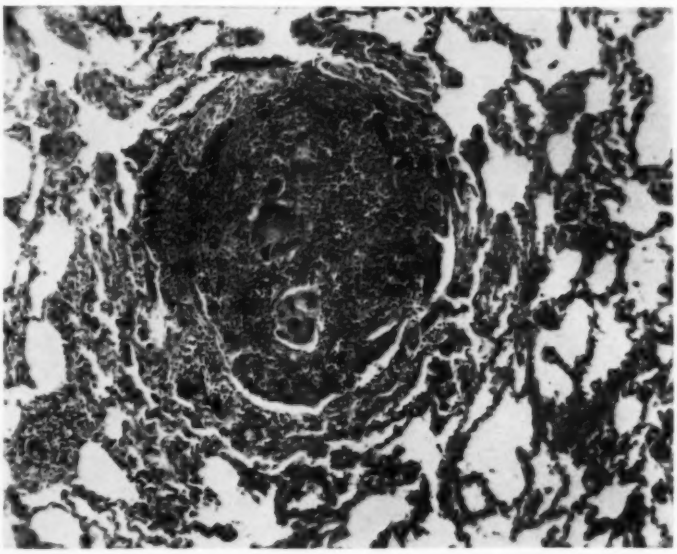


FIG. 7. Section of lung from monkey 6½ months after *S. japonicum* infection showing a pseudo-tubercle with living miracidia inside the eggs. ×100

in the blood vessels with worms were seen. In the lungs, these pseudo-tubercles were scattered thus forming focal pneumonitis.

A monkey died in August 1948, about three years after infection, from unknown cause. This monkey was infected with 200 motile cercariae in Japan. On autopsy, minimal scarring of the liver was seen, the other organs were essentially normal. Microscopic examination revealed that there were early cirrhotic changes in the liver, with fibroblast reaction and collagen fibers mostly around the periportal

areas. Parenchymal cells of the liver appeared to be normal. No pathological changes were seen in the spleen, lungs, kidney, etc.

In March 1949, approximately $3\frac{1}{2}$ years after infection, all living monkeys were subjected to liver biopsy by laparotomy. During operation no monkey showed any free fluid in its peritoneal cavity. The spleen appeared normal in all. The liver of a few of the monkeys showed

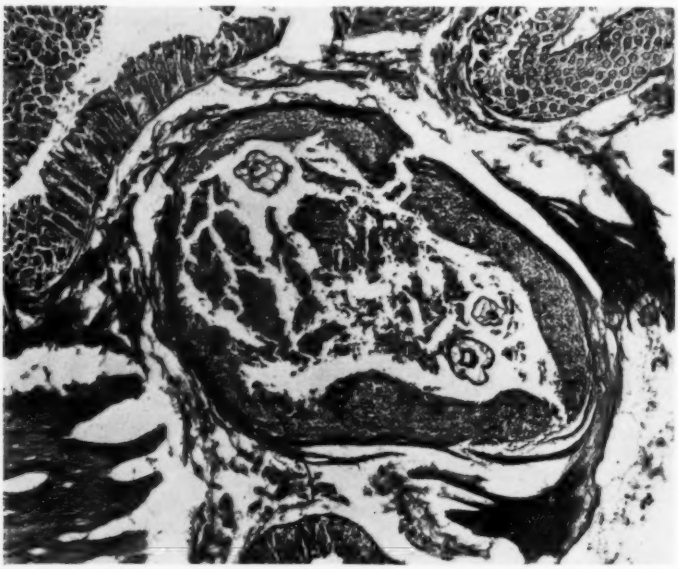


FIG. 8. Section of intestine from monkey 3 months after *S. japonicum* infection showing schistosome worms in a thrombus of mesenteric vessel.

a few small yellow nodules but none had cirrhotic changes or scarring. A piece of liver tissue from the left lobe was removed from each monkey. A lymph node from the mesenteries was also taken. In fresh preparations, by pressing the liver tissue between two slides, living or dead schistosome eggs were found in eight out of nine monkeys examined. Both *S. japonicum* and *S. mansoni* eggs were found in the doubly infected monkeys. On microscopic sections, there was again no appreciable cirrhosis. Scattered pseudo-tubercles with eggs were, nevertheless, found in each instance, with changes similar to those killed

during the acute stage of the disease. The number of eosinophiles in the pseudo-tubercles of the acute cases and chronic cases were approximately equal. We were unable to differentiate between the acute and chronic cases by examining the liver sections. No schistosome eggs were found in any of the lymph node sections of this series of biopsies.

Subsequently, one monkey died in September 1949, about four years after infection, from a cerebral concussion when he was beaten by other monkeys in the same cage. At autopsy, three pairs of worms were found in the mesenteric vessels, but no new pathological findings in the organs were found. Microscopic sections showed eggs in the liver without cirrhosis. The changes were similar to those seen at liver biopsy done six months previously.

DISCUSSION

The observations on these experimental monkeys infected with a single and light dose of *Schistosoma japonicum* appear to shed some light on the clinical problem of schistosomiasis in mild infections. Many of the veterans who contracted this disease in an endemic area and subsequently returned to the United States without further re-exposure might have had a course of infection similar to these monkeys, despite repeated treatments. In our recent survey of thirteen veterans and prisoners of war, in which seven had proven schistosomiasis 5–6 years previously, three showed continued active infection diagnosed by rectal biopsies. (12) The constant demonstration of typical mature eggs, even by a simple water sedimentation technique, either in the first or second positive stool examinations in our monkeys and dogs makes it unlikely that a diagnosis of acute mild schistosome infection need rest on the demonstration of immature forms of eggs. This is, moreover, dangerous since there are many components in the stool such as vegetable cells which may be confused with the atypical schistosome eggs. The irregular pattern of egg production with "shower" like appearance and the lack of an effect from change in diet, can probably be explained by the fact that the adult schistosomes live in the portal and the mesenteric veins. They do not lay their eggs directly into the intestinal lumen. The eggs have to be discharged into the intestinal lumen through eroded mucosa. The erosion of the intestinal mucosa is mainly due to the irritation from the eggs and their

toxic secretions and not from the food intake, as all kinds of food are pretty well digested in liquid form when they come to the small intestine. The discharge of eggs through such mucosal erosions explains the fact that we sometimes find more eggs in a piece of mucous blood fleck than in a whole stool specimen. Thus, a direct smear of any bloody mucous in a routine stool examination for schistosomiasis should always be made. It is also apparent that frequent stool examinations at intervals are necessary. This has been stressed in studies of infections of man (9, 13).

Although all the eight monkeys that are still alive to date are maintaining an active schistosome infection, the results from our serial stool examinations at different intervals suggest that their egg output is decreasing during the past four years. Direct smear and water sedimentation method would usually give positive results in the first two years. Later on, it was not easy to find eggs from their stools by these methods after two years of infection. Quantitative AEX method seemed to be satisfactory at this period, but again became insufficient to demonstrate the eggs at the end of three years of infection. 10% ethyl alcohol sedimentation method appears to be satisfactory from that time on up to date. It is difficult to make a definite statement about the actual percentage of drop of the egg output in these infected monkeys, as none of the stool examination methods we used will give an absolute quantitative count. Whether this egg reduction is due to decreasing number of worms in the host body, or due to increased fibrous tissue reaction around the eggs to prevent their discharging into intestinal lumen, or due to both factors remains to be solved.

The pathological changes due to *Schistosoma japonicum* in our experimental monkeys are similar to those found by other workers. (14, 15, 16, 17, 18) Most investigators agreed that the adult schistosome and its eggs, besides acting as a foreign body to the tissue of the host, also liberate a toxic substance which accounts for the eosinophilic and neutrophilic reaction.

In this study, the number of eosinophiles in the pseudo-tubercles of the early cases is not appreciably greater than those of the chronic infections. There are less eosinophiles around the dead calcified eggs but great numbers are found surrounding those with living miracidia. This agrees with Fairley's findings (14) which indicated that the living

miracidia within the eggs evoked the eosinophilic response. We have not seen the rays of mucoid material surrounding the egg which is believed to be the secretion from the miracidia (15).

The lack of an appreciable cirrhosis of the liver in all but one of our monkeys was a surprise to us. Eggs have been constantly discharged into the liver in sufficient number so that living eggs were demonstrated at each biopsy. Yet, the average duration of the life span of these eggs does not exceed three weeks. (19) Whether the cirrhosis of liver, which so frequently accompanies chronic schistosomiasis in man, is due to liver damage plus a dietary deficiency remains to be tested.

Liver function studies were contemplated on our monkeys, but owing to the fact that the normal level of liver function tests in monkeys is unknown, this study was not carried out. These might well have been normal.

In this study, we had only one monkey out of three show eggs in the lungs in the acute stage of the disease. Most of the remaining monkeys are still living and we are not able to draw any conclusion about the incidence of pulmonary complication of *S. japonica* in experimental monkeys.

SUMMARY

1. Fifteen *Macacus philippinensis* monkeys were infected with 100–200 motile cercariae of *Schistosoma japonicum* intra-peritoneally. Three of them were killed and autopsied during the acute stage of their illness. Eight surviving monkeys were kept alive for experimental observations for more than four years.

2. During the acute stage of the disease, by water sedimentation technique, typical matured eggs could be demonstrated in all cases by the second positive stool examination with an average incubation period of 36–37 days.

3. Daily stool examinations by quantitative acid-ether-xylol method showed no regular pattern of egg production. Sudden changes in the diet did not affect the production of eggs.

4. All of the surviving monkeys, which had been infected with *S. japonicum* 4½ years previously, were demonstrated to still be infected, but the eggs seem more difficult to detect.

5. During the acute stage of the disease, autopsy of three monkeys

showed eggs in the liver and intestines. In one monkey, eggs were also found in the lungs. The pathological changes are mainly a type of foreign body and pseudotubercle reaction around the eggs, with mononuclear, polymorphonuclear, and eosinophilic cellular infiltration.

6. One monkey, which had an infection produced by 200 cercariae, died three years after infection. There were early minimal cirrhotic changes in the liver, in addition to pseudo-tubercle reactions around the eggs.

7. Liver biopsy of nine monkeys, 3½ years after the infection, showed eggs in the liver, with pseudo-tubercle and foreign body reaction as in the acute cases, and no appreciable scarring or residues of old degenerate eggs.

We wish to express our appreciation and indebtedness to Dr. N. G. Hairston for much of the data during the early period of the infection; to Mr. B. Papirmeister and Mr. C. Wieneke for technical help; to Dr. C. T. Li for the liver biopsies; to Mr. L. Douglass for care of the monkeys and Mr. C. Reather for photography.

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CHARACTERISTICS OF THE GRADIENTS OF UTERINE CONTRACTILITY DURING THE FIRST STAGE OF TRUE LABOR*

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Received for publication January 25, 1950

In a previous paper (1) utilizing the multi-channel strain-gage tokodynamometer, the concept was enunciated for the first time that progressive labor is the result of an increasing gradient of work from the fundus to the lower uterine segment. The realization that gradients of activity were physiologically important for cervical dilatation clarified much, not only in regard to normal labor, but to false and abnormal labor as well. Nevertheless, certain facts regarding the characteristics of these gradients remained obscure since the initial impression was based upon observations on only a few subjects. In order to study the magnitude and variability of the contraction characteristics and to have some idea of their progressive change throughout the first stage of labor, as well as to determine whether the gradients differed from multiparas to primiparas, it became necessary to study a large number of normal patients throughout labor and to submit the data thus obtained to a statistical analysis. This is the report of such a study.

The first stage of labor in 151 normal women was analyzed with the tokodynamomemter. The entire procedure was explained thoroughly to these patients and every effort was made to avoid artefacts and complicating situations, such as patient movement, eating, and undue obesity. Every effort was made to standardize the gages† of the

* Aided in part by a grant from the Pharmaceutical Division, Sandoz Chemical Works, New York, to The Johns Hopkins University School of Medicine.

We gratefully acknowledge our debt to Jane B. Holt who recorded from all patients and analyzed all records.

† A standard procedure was employed by hanging a 50 gram weight on the lever arm of the strain gage pick-up and adjusting the current strength through the gage so that a deflection of a given amount was obtained. Since the method of

tokodynamometer according to the method previously described in order that the results obtained would be comparable. Each patient's chart was reviewed at the completion of her labor in order to determine the duration of the first stage and the normality of the process. Records in which these items were in doubt were discarded. The tracings were then divided into twenty-minute intervals, and the average intensity of the contractions in grams, the average duration of the contractions (exclusive of relaxation) in seconds, the contraction time in seconds and the frequency were recorded. From these data, the hourly rate of work was calculated. Inasmuch as labor is a dynamically progressive function, the resulting figures were found to change from one twenty-minute period to the next throughout labor. It was thus reasoned that each twenty-minute period could be considered a separate observation, and so, although 151 patients were studied, 677 separate periods of observations were recorded. This latter figure was used in calculating the standard error of the means. To comprehend further the progressive change in these figures, the first stage of labor was arbitrarily divided by time into thirds, each third then being compared with the other.

These data, together with pertinent information regarding the patient and her labor, were recorded on code sheets (Figure 1) and transferred to punch cards. Thus, the final sorting and correlation of data from the 600 odd observations was greatly facilitated. Table I, section A, summarizes the information concerning the number of patients, the number of observations, the average duration of each third of labor, and the average cervical dilatation during each third. Sections B, C, D, E, and F record respectively, at three uterine levels (fundus, mid-uterus, lower uterus) the mean intensity of the contractions, the mean duration of the contractions, the mean contraction time, the frequency of contraction, and the hourly rate of work. The standard errors of the mean are recorded, and thus, by a simple mathematical formula the significance of the difference between the means in the various thirds of labor can be tested.

These data are portrayed graphically in Figures 2 through 5. In

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suspending the weight affects importantly the deflection on the recorder, our intensity of contraction figures must be regarded as arbitrary and relative rather than absolute.

FIGURE 1

THE JOHNS HOPKINS HOSPITAL

Department of Obstetrics

Department of Embryology, Carnegie Institution

TKD STUDY

NAME:

COL.		COL.		COL.	
1–6	HISTORY NUMBER	25–26	CONTRACTION INTENSITY in Gms.-Fundus	41–42	DURATION of CONTRACTION in SECONDS-LOWER SEGMENT
7–10	CARD-NUMBER	27–28	CONTRACTION INTENSITY in Gms.-Miduterus	43–44	RATE of CONTRACTION in Gms./Sec.-Fundus
11–12	NUMBER of CARDS	29–30	CONTRACTION INTENSITY in Gms. Lower Segment	45–46	RATE of CONTRACTION in Gms./Sec. Miduter.
13–14	ANALYSIS NUMBER	31–32	CONTRACTION TIME in SECONDS Fundus	47–48	RATE of CONTRACTION in Gms./Sec. Lower Seg.
15–16	PERCENTAGE of LABOR First Stage	33–34	CONTRACTION TIME in SECONDS-MIDUTERUS	49	LABOR DIAGNOSIS 0 True Labor 1 False labor 2 Prodromal labor 3 Uterine inertia, comp. 4 Uterine inertia, a 5 Uterine inertia, b 6 Postpartum Y Not in labor
17–18	PERCENTAGE of LABOR Second Stage	35–36	CONTRACTION TIME in SECONDS-Lower Segment		
19–20	DILATATION of CERVIX Average Cms.	37–38	DURATION of CONTRACTION in SEC.-Fundus		
21–22*	FREQUENCY of CONTRACTIONS-Aver./20 Min.	39–40	DURATION of CONTRACTION in SECONDS-MIDUTERUS		
23–24	FREQUENCY of CONTRACTIONS-Rate/Hr.				

* Punch Y Col. 21 if other than 20 minute interval is averaged.

FIGURE 1—*Continued*

COL.	COL.	COL.
50 DRUG to AFFECT LABOR	51 ANALGESIC	52 ANALGESIC
Y None	Y None	Y None
1 Pituitrin, intermittent	1 Demerol	53–54 DURATION of 1ST STAGE
2 Pituitrin, intravenous	2 Demerol-Barb.-Scop.	Labor Hours—TOTAL
3 Pitocin, intermittent	3 Demerol and Scopolamine	55–56 LABOR HOURS
4 Pitocin, intravenous	4 Barbiturate	FIRST STAGE
5 Calcium	5 Barbiturate & Scopolamine	57–58 LABOR MINUTES
6	6 Scopolamine	SECOND STAGE
7	7 Spinal	59–60 LABOR MINUTES
8	8 Caudal	THIRD STAGE
9	9 Morphine	
X	0	
	X	

addition, the graphs contain the curve of the frequency of contraction and the curve of cervical dilatation. The dotted lines represent extrapolations of the respective curves. In the case of the frequency of contraction curve, however, the figures for the first ten percent of the first stage of labor were actually determined from the records.

Tables II and III are roughly similar to Table I except that they divide the data according to primiparous or multiparous types of labor. In both of these tables, section A again summarizes the information concerning the number of patients, the number of observations, the average duration of each third of labor, and the cervical dilatation during the respective third. Sections B, C and D record the respective mean intensity, mean duration of contractions, and hourly rates of work at three uterine levels. A comparison between Tables II and III will show some of the different characteristics of uterine contractility between multiparas and primiparas.

COMPOSITE CHANGES, FIRST STAGE OF LABOR

Examination of Table I shows marked fundal predominance in each third of labor, in intensity and duration of contraction, and contraction time for all subjects. In each instance, the fundus demonstrates

greater activity than does the mid-uterus or the lower uterine segment. Examination of the standard error of the mean attests the significance of this gradient of fundal dominance. If the behavior of the fundus

TABLE I

Summary of data of characteristics of uterine contractility during the first stage of true labor during 677 twenty-minute periods of observation in 151 women

A. *Correlative Information*

PER CENT OF FIRST STAGE	NUMBER OF CASES	NUMBER OF OBSERVATION PERIODS (20 MINUTES EACH)	AVERAGE DURATION	AVERAGE CERVICAL DILATION
%			*hours*	*cms*
0–33.3	39	148	5.1	2.7
33.4–66.6	57	229	5.3	3.6
66.7–100	55	300	4.6	5.9

B. *Intensity of Contractions in gms./average contraction*

PER CENT OF FIRST STAGE	FUNDUS		MID-UTERUS		LOWER UTERUS	
	Grams	S.E.M.	Grams	S.E.M.	Grams	S.E.M.
%						
0–33.3	32.9	±1.52	19.9	±1.09	1.70	±0.27
33.3–66.6	34.4	±0.93	24.2	±0.83	1.06	±0.25
66.6–100	41.3	±0.92	23.3	±0.01	1.20	±0.21

C. *Duration of Contraction (excluding relaxation phase)*

PER CENT OF FIRST STAGE	FUNDUS		MID-UTERUS		LOWER UTERUS	
	Seconds	S.E.M.	Seconds	S.E.M.	Seconds	S.E.M.
%						
0–33.3	38.3	±1.82	29.5	±1.19	4.2	±0.81
33.3–66.6	42.7	±1.09	30.6	±1.10	2.2	±0.47
66.6–100	44.1	±0.89	30.4	±0.96	1.8	±0.86

D. *Contraction Time*

PER CENT OF FIRST STAGE	FUNDUS		MID-UTERUS		LOWER UTERUS	
	Seconds	S.E.M.	Seconds	S.E.M.	Seconds	S.E.M.
%						
0–33.3	28.5	±0.75	24.2	±0.83	3.3	±0.57
33.3–66.6	29.7	±0.63	23.2	±0.77	1.8	±0.35
66.6–100	30.8	±0.57	24.2	±0.73	1.5	±0.28

TABLE I—*Continued*

E. *Frequency in Contractions/hr.*

PER CENT OF FIRST STAGE	FREQUENCY
%	
0–33.3	19.1 ± 0.69
33.4–66.6	20.1 ± 0.61
66.7–100	21.0 ± 0.46

F. *Hourly Rate of Work in Dynes/hour* × 10^3

PER CENT OF FIRST STAGE	FUNDUS	MID-UTERUS	LOWER UTERUS
%			
0–33.3	130	54	0.7
33.3–66.6	169	72	0.22
66.6–100	285	75	0.21

alone be examined, then Table I demonstrates that the fundus shows progressively greater intensity of contraction, a longer duration of contraction, and a longer contraction time as the first stage of labor progresses. Thus, the fundus shows an average intensity of contraction of 32.9 grams in the first third of the first stage of labor and 41.3 grams in the last third of the first stage. These differences are highly significant. There is a slight, but significant, change in intensity of contraction of the mid-uterus throughout labor, and a suggestive change in duration of contraction. The contraction time of the mid-uterus, however, is unchanged. The changes taking place in the contractility pattern of the mid-uterus throughout the first stage are, however, of much less magnitude than those of the fundus. Thus, fundal advantage over the mid-uterus increases progressively throughout the first stage of labor. This is shown quite well in the graphic reproduction of these figures shown in Figures 2 through 5. As the fundal advantage becomes more apparent dilatation of the cervix is speeded. This, too, appears in the graphs and is a well known clinical fact. Quite the reverse of the fundal pattern is seen in the lower uterine segment. Here, the activity becomes less and less, and while the differences are small they seem, from a statistical viewpoint, to be significant. This again adds to the fundal advantage as labor progresses. One further point needs attention. As the fundus contracts with greater vigor, and as the contrac-

tions are prolonged, the contraction time becomes prolonged. Thus, a gradual rise to maximum contractility may make a more efficient

TABLE II

Summary of data of characteristics of uterine contractility during the first stage of true labor in sixty-eight primiparas

A. *Correlative Information*

PER CENT OF FIRST STAGE	NUMBER OF PATIENTS	NUMBER OF OBSERVATIONS	AVERAGE CERVICAL DILATATIONS	AVERAGE DURATION
%				*hrs.*
0–33.3	21	90	2.7	8.8
33.4–66.6	29	115	3.3	6.2
66.7–100	18	97	6.9	4.2
Total	68	302		19.2

B. *Intensity of Contractions in gms./average contraction*

PER CENT OF FIRST STAGE	FUNDUS		MID-UTERUS		LOWER UTERUS	
	Grams	S.E.M.	Grams	S.E.M.	Grams	S.E.M.
%						
0–33.3	34.01	±1.16	21.35	±1.32	2.30	±0.42
33.4–66.6	32.99	±1.12	21.49	±1.54	0.53	±0.00
66.7–100	38.98	±1.47	21.95	±1.66	1.18	±0.35

C. *Duration of Contraction in Seconds (excluding relaxation phase)*

PER CENT OF FIRST STAGE	FUNDUS		MID-UTERUS		LOWER UTERUS	
	Seconds	S.E.M.	Seconds	S.E.M.	Seconds	S.E.M.
%						
0–33.3	37.93	±1.17	31.86	±1.53	6.30	±1.27
33.4–66.6	40.83	±1.42	28.09	±1.91	1.32	±0.41
66.7–100	42.84	±1.36	28.94	±1.85	2.49	±0.86

D. *Hourly Rate of Work in Dynes/hour* $\times 10^3$

PER CENT OF FIRST STAGE	FUNDUS	MID-UTERUS	LOWER UTERUS
%			
0–33.3	125	64	1.40
33.4–66.6	173	69	0.06
66.7–100	193	70	0.38

contraction in the smooth muscle of the uterus. It is interesting to note that with prolongation of contraction time of the fundus, there

is a corresponding diminution in this time in the lower uterine segment, throughout the first stage of labor.

TABLE III

Summary of Data of Characteristics of Uterine Contractility During the First Stage of True Labor in Eighty-three Multiparas

A. *Correlative Information*

PER CENT OF FIRST STAGE	NUMBER OF PATIENTS	NUMBER OF OBSERVATIONS	AVERAGE CERVICAL DILATATIONS	AVERAGE DURATION
%				*hrs.*
0–33.3	18	58	2.86	2.7
33.4–66.6	28	114	4.30	3.9
66.7–100	37	203	5.19	5.4
Total	83	375	12.35	

B. *Intensity of Contractions in gms./average contraction*

PER CENT OF FIRST STAGE	FUNDUS		MID-UTERUS		LOWER UTERUS	
	Grams	S.E.M.	Grams	S.E.M.	Grams	S.E.M.
%						
0–33.3	31.17	±2.18	18.36	±2.04	0.57	±0.00
33.4–66.6	36.00	±1.29	23.00	±0.99	1.59	±0.49
66.7–100	42.62	±1.23	23.93	±1.09	1.18	±0.25

C. *Duration of Contraction in Seconds (excluding relaxation phase)*

PER CENT OF FIRST STAGE	FUNDUS		MID-UTERUS		LOWER UTERUS	
	Seconds	S.E.M.	Seconds	S.E.M.	Seconds	S.E.M.
%						
0–33.3	38.88	±2.05	27.19	±1.56	1.00	±0.35
33.4–66.6	44.65	±1.56	33.70	±1.39	3.09	±0.83
66.7–100	58.11	±1.41	31.38	±1.14	1.64	±0.41

D. *Hourly Rate of Work in Dynes/hour* $\times 10^8$

PER CENT OF FIRST STAGE	FUNDUS	MID-UTERUS	LOWER UTERUS
%			
0–33.3	140	49	0.04
33.4–66.6	181	90	0.5
66.7–100	362	79	0.2

It should be noted, however, that most subjects in labor fail to exhibit any activity whatsoever in the lower part of the uterus. In fact,

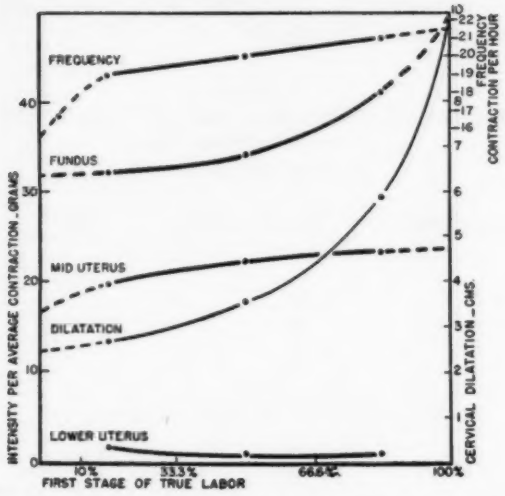

FIG. 2. Comparison of the intensity per average uterine contraction between the fundus, mid-uterus and lower uterus in the first stage of labor.

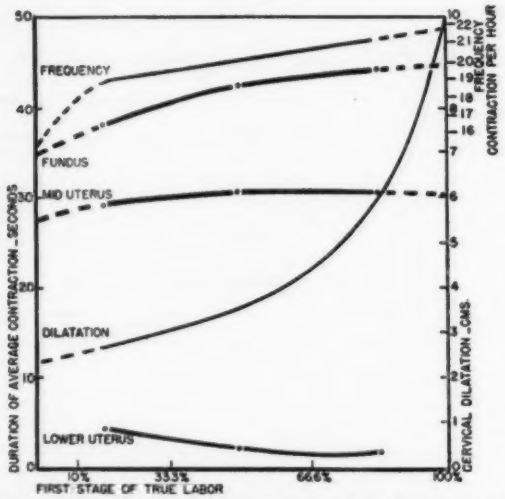

FIG. 3. Comparison of the duration of contraction between the fundus, mid-uterus and lower uterus in the first stage of labor.

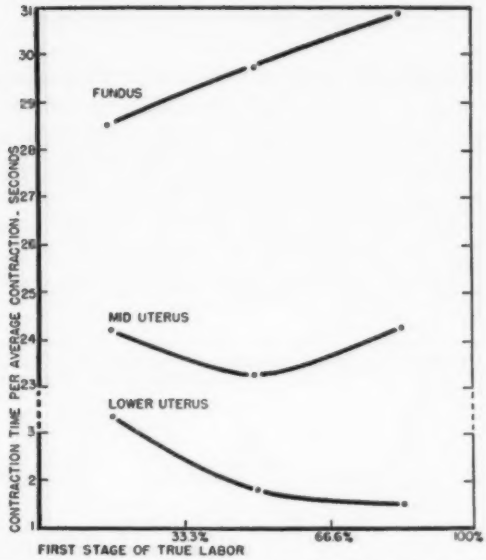

FIG. 4. Comparison of the contraction time between the fundus, mid-uterus and lower uterus in the first stage of labor.

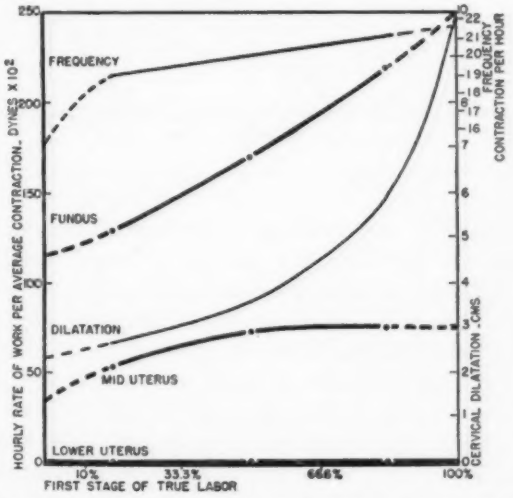

FIG. 5. Comparison of the hourly rate of work between the fundus, mid-uterus and lower uterus in the first stage of labor.

the data in this regard may be summarized as follows: In the first third of the first stage of labor 75.5% of primiparous and 93.3% of multiparous women fail to show activity in this part of the uterus; in the last two thirds of the first stage of labor, about 90% of gravidas have inactive lower uterine segments, and in those who do exhibit activity, it is inappreciable when compared with the activity of the fundus.

The changes in the intensity of the contractions of the various segments during the first stage, and the comparison of the different

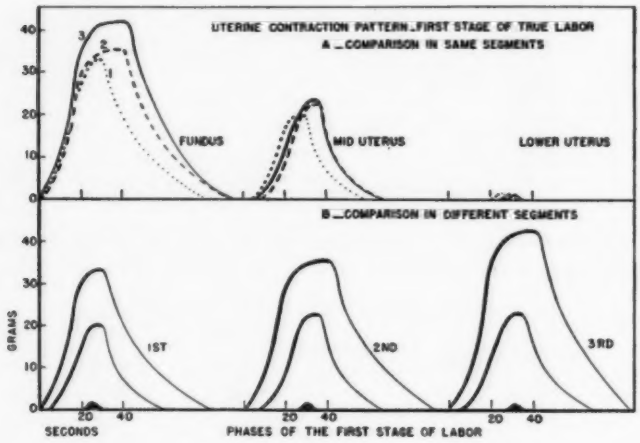

FIG. 6

segments during the various thirds of this stage are shown in Figure 6, which brings out the increasing gradient of fundal dominance very well. The amplitude of this gradient is well portrayed in the same figure. Thus, the fundus gains in power during the first stage with little, or no, increase in activity of the mid-uterus and lower segment. The increase in the increment of fundal activity over that of the mid-segment is nicely shown in the lower half of Figure 6. When the hourly rate of work is calculated, as shown in section E, Table I, the relative increase in fundal work over the mid-uterus is quite apparent, while the work done by the lower uterine segment shows an actual decrease.

THE COMPARISON OF CONTRACTION CHARACTERISTICS IN MULTIPARAS AND PRIMIPARAS

Tables II, III and Figure 7 demonstrate the difference in contractility patterns between multiparas and primiparas. During the first third of the first stage, the fundus in the primiparous woman contracts with greater intensity than that in the multiparous woman. However, as labor progresses the multiparous fundus begins to work with greater intensity than that in the primipara. The duration of contraction of

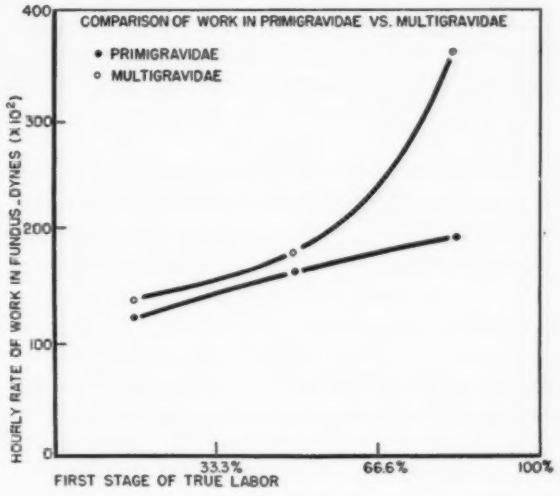

FIG. 7

the fundus is greater in the multipara in each third of labor. The same phenomenon is present in the mid-uterus although to a much less marked degree. In the primipara the lower uterine segment shows a descending degree of activity, while in the multipara, there is a tendency to reversal of this situation. However, when the hourly rate of work is calculated it becomes apparent that in all instances the fundus of the multipara does more work than that of the primipara.

The phenomenon of an increased gradient of activity is perhaps best

demonstrated by Table IV in which the work index of the first third and last third of labor in primiparas and multiparas is compared. The work index is an arbitrary figure obtained by dividing the hourly rate of work performed by the fundus by that performed by the mid-uterus during the same period of time. It is obvious that this figure is a coefficient expressing the gradient of activity from the fundus to the mid-uterus and it is an index of fundal dominance of the more caudal part of the uterus. The more this figure exceeds one, the greater is the magnitude of fundal dominance. Furthermore, one of the characteristics of normal labor is an increase in the value of the work index as labor progresses. The greater this increase, the more efficient and more rapid the labor. It will thus be seen that the work index increases both in the multiparas and in the primiparas. However, the increase between the first and third thirds of the first stage of labor in multiparas is 60.1 percent, while that in primiparas is but 41.5 percent.

TABLE IV

The work index in the first and last third of the first stage of normal labor showing percentage increment

	FIRST THIRD	LAST THIRD	PER CENT INCREMENT
Primipara	1.95	2.76	41.5
Multipara	2.86	4.58	60.1

RELATION TO PREVIOUS OBSERVATIONS

Various methods have been used to measure the characteristics of uterine contractility during labor in the past (2). These may be divided into two general categories, namely, the use of external tokographs and the use of internal balloons or direct reading from the amniotic sac. Inasmuch as all of the external tokographs differ radically in principle from the instrument used in this paper, and inasmuch as they obtain their information from one lead, they obviously do not give the same type of information. The internal methods of measurement are, by and large, irritative and can only measure intrauterine pressure exerted against a receptor in one location regardless of the source of pressure within the uterus. This type of information, while valuable, differs

obviously from that which has been obtained from the multichannel strain gage instrument recording as it does simultaneously from three different levels in the uterus. There have been, until recently, no other data indicating the existence of gradients of activity from the fundus to the lower uterine segment during labor. Fortunately, however, Karlson utilizing a small intrauterine receptor at three locations in the uterus, namely, the fundus, the mid-uterus and the cervix, has recently (3) confirmed, in a small series of cases, the presence of a decreasing gradient of force during labor. This confirmation, obtained with an entirely different instrument and different method of measurement, clearly confirms the conclusions which have been drawn from the data cited above. Moreover, observations on the bioelectric potentials of uterine origin likewise confirm the existence of a diminution gradient of muscular activity during the first stage of labor (4).

SUMMARY

1. Sixty-eight primiparas and eighty-three multiparas were subjected to multichannel tokodynamometric studies during the first stage of normal labor. Six hundred and seventy-seven separate twenty-minute periods of observation were made on these patients.

2. A decreasing gradient of activity was found from the fundus to the lower uterine segment in both multiparas and primiparas. Thus, the fundus contracted more strongly and had a contraction of greater duration and of greater efficiency than the mid-uterus or the lower uterine segment throughout labor.

3. The increment of fundal dominance increased throughout labor both in primiparas and multiparas, but more so in the latter than in the former. The percentage increase in primiparas was 41.5 percent, while that in multiparas was 60.1 percent. The greatest increase in fundal dominance occurred in multiparas after the cervix was dilated about 5 centimeters. Such an increase after 50% dilatation of the cervix is not observed in primiparous women.

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THE SUPPRESSION OF ANDROGEN SECRETION BY CORTISONE IN A CASE OF CONGENITAL ARDENAL HYPERPLASIA[1]

Preliminary Report

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Received for publication February 13, 1950

In congenital hyperplasia of the adrenal the major clinical manifestations are due to excessive secretion of androgens. Unsuccessful attempts have been made by us (unpublished) to suppress the secretion of 17-ketosteroids in patients with this disorder by the administration of steroids, such as 17-ethyl testosterone, 17-vinyl testosterone, 17-methyl androstenediol and 17-methyl androstanediol, which have a chemical structure similar to that of androgens but possess relatively little androgenic activity. However, 17-vinyl testosterone was found to cause a significant decrease in size of the rat adrenal (1).

Cortisone (11-dehydro-17-hydroxycorticosterone) was administered to a patient with congenital adrenal hyperplasia in the hope that a hormone of this type which is essential for normal metabolism, if given in quantities adequate for or greater than the normal needs of the body, might suppress the endogenous activity of the adrenals including the hypersecretion of androgen.

The patient, P. R. (H.L.H. A 58433), was a female pseudohermaphrodite 15 years of age who had been under observation for eleven years. In 1941, at the age of $6\frac{1}{4}$ years, her left adrenal and a portion of the right were removed by Dr. Hugh H. Young without influencing the progressive virilization and hirsutism which occur regularly in

[1] This work was made possible by a grant from the American Cancer Society for studies on the relationship of the pituitary hormones, thyroid hormone and the steroid hormones of the adrenal glands and gonads to normal and abnormal growth, and by a grant from the Commonwealth Fund for the study of endocrine problems in childhood.

this disease. During the seven years before this study, repeated determinations of the urinary 17-ketosteroids showed daily excretions ranging from 20 mg. to 68 mg. per day, with an average of 42 mg. per day.

After a control period of 10 days, during which the urinary 17-ketosteroids were measured daily and the 11-oxysteroids every other day, treatment with cortisone crystals in aqueous suspension (Merck —Lot No. 9R7184) was given intramuscularly at 6 hour intervals in doses amounting to 100 mg. daily for a period of 15 days.

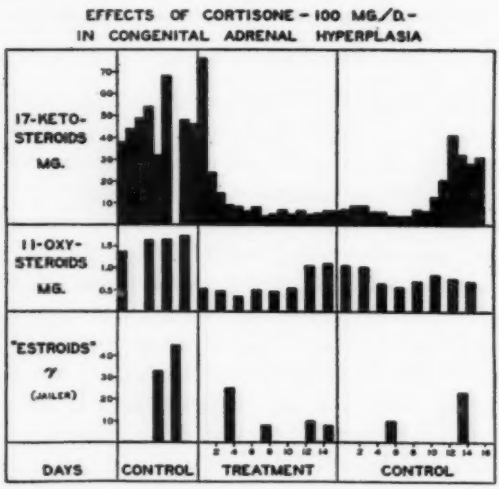

FIG. 1

As shown in the chart, the excretion of 17-ketosteroids during the control period varied between 32 and 68 mg. per day, with an average of 48 mg. per day. It decreased sharply on the second day of treatment and after the 4th day remained at a level between 4.0 and 8.4 mg. per day, with an average of 6.5 mg. per day, throughout the 15 days of treatment. The output did not increase until 10 days after treatment was discontinued and then rose to levels between 28 and 41 mg. per day.

The excretion of 11-oxysteroids, determined by the method pre-

viously described (2), varied between 1.39 and 1.74 mg. per day, with an average of 1.61 mg. per day, before treatment. On the first day of treatment the output decreased to 0.58 mg. per day and during the first 10 days of treatment remained between 0.37 and 0.51 mg., with an average of 0.47 mg. On the 12th and 14th days of treatment the oxysteroid excretion increased to 1.04 and 1.07 mg. respectively. On discontinuing treatment, the output gradually decreased to 0.58 mg. by the 7th day, rising to levels of 0.67 to 0.83 mg. at the end of the study.

The urinary estroids, determined by the fluorometric method of Jailer (3), followed the same pattern as the 17-ketosteroids. They decreased from a pretreatment level of 33–44 micrograms to levels of 8–10 micrograms during treatment with cortisone. On the 6th day after discontinuing treatment, the output was 10 micrograms but on the 13th day it had risen to 23 micrograms.

Metabolic balance studies made during the experiment do not permit an accurate interpretation because the patient refused at times portions of the fixed diet which had been prescribed. There was no alteration in the concentration of the serum electrolytes, which were normal throughout the study. No significant change in the glucose tolerance curve was observed. The blood pressure varied between 98/70 and 126/98 before treatment and 100/70 and 120/80 during treatment. The eosinophil count throughout both the control period and treatment period fluctuated between 67 and 155 per c. mm., with an average of 92 per c. mm., except that one count was 333 per c. mm. on the 2nd day of treatment. No plethora was observed. During the short period of observation no changes in hirsutism or other clinical signs were detected.

CONCLUSIONS

It is suggested that in this case of congenital adrenal hyperplasia, cortisone in doses of 100 mg. daily suppressed the secretion of the adrenal hormones responsible for the urinary excretion of 17-ketosteroids, 11-oxysteroids and estroids. In respect to 17-ketosteroids and estroids there was no tendency for this effect to pass off during the 15 days of treatment and the suppression lasted for 11 days after discontinuing treatment. The output of 11-oxysteroids was suppressed

during the first 10 days of treatment but rose during the latter part of treatment, possibly due to the excretion of a portion of the cortisone administered. Further studies are necessary in this and other cases to determine the minimal dose of cortisone which will suppress androgen secretion and to learn whether this effect can be maintained for long periods. It is believed that this drug may prove of therapeutic value.

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MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HURD HALL, THE JOHNS HOPKINS HOSPITAL, MONDAY,

JANUARY 9, 1950—8:15 p. m.

Dr. Wilkins: The first paper this evening is by Dr. Jonas Friedenwald and Dr. Robert Day, and will be presented by Dr. Friedenwald.

THE VASCULAR LESIONS OF DIABETIC RETINOPATHY

With increasing life span of diabetics there is an alarming increase in the incidence of so-called degenerative complications of diabetes. Statistical reports from many clinics indicate that the retinopathy is related to the duration of the diabetes rather than to the age of the patient. Its incidence is not significantly related to the severity of the diabetes nor to the rigors nor methods of treatment.

Histologic studies of the retina reveal that in many cases of diabetic retinopathy the retinal arteries show neither atherosclerosis nor hyalin degeneration of their media. The characteristic vascular lesion to be found in the retina in these cases consists in the presence of great numbers of saccular capillary aneurysms, often with hyalinized walls. Such capillary aneurysms are not regularly found in cases of generalized atherosclerosis nor of malignant hypertension, though, as recently demonstrated by Becker and Post, saccular capillary aneurysms associated with fusiform capillary varicosities are to be found after retinal venous occlusion. The lesion in the retina of diabetics is closely related to that first described in the kidney by Kimmelstiel and Wilson. Other organs have not as yet been adequately explored.

The retinal lesions can be beautifully demonstrated with the aid of the Hotchkiss-MacManus stain for mucoids. This stain reveals the presence of a vascular basement membrane lying beneath the endothelium in arterioles, capillaries and veins of the retina. In unsectioned flat preparations of the retina stained by this means the whole retinal vascular pattern becomes clearly visible and, in diabetics the relation of the capillary aneurysms to the vascular net is well seen. Hemorrhages and exudates frequently surround the aneurysm indicating that weaknesses in the vessel wall are associated with the aneurysms.

Dr. Wilkins: I would like to ask Dr. Rich if he has any comment to make upon this "old" discovery of Dr. Friedenwald.

Dr. Rich: The chief comment I would like to make is one with which I am sure you will agree, and it is that this is a very beautiful observation. Dr. Friedenwald has contributed so much in the past to the pathology and pathological physiology of the eye, and this present observation is something very special, in spite of the fact that, as Dr. Friedenwald tells us, Mackenzie may have seen these aneurysms in the last century.

The relationship with the Kimmelstiel glomerular lesion is extremely interesting. I wonder, Dr. Friedenwald, whether capillary aneurysms may not occur more frequently in the brain and meninges than your studies of those tissues suggest. The number of capillaries and the extent of the capillary bed visible in a microscopic section of the brain are much less than in a spread of the retina. It may well be that comparable methods applied to the brain and meninges may reveal more capillary angiomata than examination of random sections may lead one to suspect.

The nature of the process responsible for the development of these remarkable little aneurysms in the retina and kidney in diabetes is a problem of the greatest interest. Evidently, something relating to the altered metabolism in diabetes is escaping us.

The finding of the ancient, prior description by Mackenzie reminds me of the rediscovery of the important miliary aneurysms of the meningeal arteries, which began to attract the widespread attention of clinicians and pathologists in the nineteen twenties, though they had been beautifully described and pictured by Gull in 1859.

Dr. Wilkins: The next paper is by Dr. Leslie N. Gay and Dr. Paul E. Carliner and will be presented by Dr. Gay.

A SUBSEQUENT REPORT ON DRAMAMINE: THE PROPHYLACTIC AND THERAPEUTIC CONTROL OF MOTION SICKNESS

In February, 1949, the preliminary report on the prevention and treatment of motion sickness with Dramamine was presented to the Johns Hopkins Medical Society. The study was planned and executed by Gay and Carliner on the U. S. A. T. General Ballou. This transport carried 1366 soldiers to Bremerhaven, Germany. The data accumulated on this voyage indicate that Dramamine in doses of 50 to 100 mg. every six hours is a powerful nontoxic, prophylactic and therapeutic drug capable of controlling the symptoms of seasickness.

During 1949 several investigations threw light on the action of Dramamine. The researches of Himwich, of Haley and Harris and of Batterman contribute to the knowledge of the drug. The usefulness of Dramamine has been confirmed by the original investigators, by the United States Navy and Army and Air Force and by four prominent commercial airlines. The consensus of observers is that Dramamine ranks as the most satisfactory drug that has been given to medical science to relieve travelers from a most distressing syndrome of symptoms.

The efficacy of Dramamine in labyrinthine disturbance has been demonstrated by Campbell, as well as by Gay and Carliner.

Dr. Wilkins: Dr. Gay's paper is now open for discussion.
Dr. Bard.

Dr. Bard: I think, first of all, I would like to say that the introduction of any substance which does not have too many side effects, and which will relieve the

horrible sensation known as nausea, is to be welcomed. I think Dr. Gay has presented some very interesting evidence indicating that he may have a means of controlling nausea and vomiting caused by a variety of conditions.

One of the questions that has been raised by this talk, one which we really, don't know much about, and yet which is very important, is the site of action of dramamine, or hyoscine, or a thiobarbiturate, all of which are effective prophylactics for motion sickness. Dr. Gay points out that they act centrally. I suspect that he is correct. Yet it is hard to believe that three drugs having such different pharmacological properties all act in the same place. We know they all have central actions and, therefore, one may suppose that they do act centrally rather than peripherally on the labyrinth. As regards the experiments of Himwich, it would be very interesting to see what would happen if he did a bilateral labyrinthectomy. That would determine whether or not his injections into the carotid acted centrally or peripherally. I might point out that hyoscine, which is quite effective in human beings, is absolutely ineffective in dogs in the control of motion sickness, whereas the thiobarbiturate known as V-12 was found to be 100 per cent effective in the most susceptible dogs. On the other hand V-12 is only moderately effective in human beings. The question is, do they act in different places?

As regards the reports that Dr. Gay has given us about tests on seagoing vessels of some size, on various airlines, etc., I think that one might ask whether any controls were done, whether placebos were given, whether the treated patients were in a place comparable to other individuals who got nothing, and what criteria were used to determine whether these people were really very susceptible or not. Then, in the case of the therapeutic use of the drug one wonders whether, if they were always sick, they kept it down. I can't fancy that an airline hostess would give it intrarectally.

There was one other point, Dr. Gay, with regard to the Navy report from Captain Shaw that you mentioned. This is one he sent me just the other day and I read it with great interest. It didn't make any difference whether the Marines had dramamine, hyoscine, placebos, or nothing; they shot equally well. This reminds me of some rather interesting work Dr. Tyler did during the war, also with Marines. He showed that a mixture of hyoscyamine and hyoscine didn't affect their shooting at all. In another rather good experiment, he got results which indicate that "MSP," a compound which contains atropine, hyoscine and sodium amytal caused them to shoot a little better, probably because of the amytal.

The drowsiness, by the way, I don't think makes any difference under ordinary commercial flying conditions, or with the fleets and the boys in landing craft. On the other hand, drowsiness is something you do worry about if you are giving a substance or substances to such individuals as members of an air crew on a long bombing mission when sleepiness, for instance, in a gunner is apt to interfere seriously with his military duties. On the other hand, a little drowsiness in infantrymen in a landing craft under fire is something I don't think you need to worry about.

Dr. Wilkins: Are there any other questions or discussion? Do the laryngologists have anything to add on the question of Meniere's disease?

Dr. Loch.

Dr. Loch: The therapeutic results with Dramamine in motion sickness reported by Dr. Gay are very convincing and I would like to add that I had equally good results with Dramamine in patients with motion sickness. One patient who had a disturbance of the otoliths improved also very well under this medication.

In Meniere's disease, the evaluation of therapeutic results is quite difficult since Meniere's disease shows spontaneous remissions. I believe it will take considerable time to evaluate the results.

I have used Dramamine following fenestration operation. The procedure was somewhat different than the one employed by Campbell. Since the labyrinthine reaction after fenestration operation is quite different in the individual patient, I feel that each patient should be used as his own control. For this reason, I have used on the same patients, alternately Placebo and Dramamine. Unfortunately for this study, many patients with the fenestration operation did not have appreciable dizziness and nausea and were, therefore, not suitable subjects for these experiments. The number of patients in which Dramamine was used is therefore much smaller than in Campbell's report. Some patients showed good results within one-half hour after Dramamine medication, but, surprisingly enough, they also showed good results after administration of Placebo. In some instances they were relieved by Placebo in a shorter time than by Dramamine. I remember, however, that three patients showed quite definite and convincing response to Dramamine and not to Placebo. Dramamine was administered to relieve the vestibular symptoms.

I have also used Dramamine in normal individuals. The caloric response was determined before and one-half hour after Dramamine was administered. There was no change of the vestibular reaction whatsoever after the Dramamine medication.

Dr. Wilkins: Are there any further questions or discussions?

Dr. Gay.

Dr. Gay: In my opening remarks this evening I stated that it is quite impossible to say an individual is going to be motion-sick because on previous trips he experienced these symptoms. Therefore, I think that all prophylaxis is open to criticism from a statistical point of view. Commercial airlines cannot use a placebo. It is against the policy of their medical directors to use passengers experimentally because of the legal complications which might follow. Dr. Bard's inquiry concerning the use of suppositories in air travel has already been considered by the commercial manufacturer. The medical directors of airlines look forward to the time when suppositories will be available in order to take care of the airsick passenger who cannot retain the drug after it is given by mouth.

Referring to the fenestration operation in conversation with Dr. Crowe and Dr. Loch, I am of the impression that one technique might produce more violent labyrinthitis than another technique. Dr. Campbell, who has performed many of these operations, apparently obtains severe labyrinth disturbance. I am not discussing his technique. I merely cite Dr. Campbell's report as data which reveal the action of Dramamine in violent labyrinth disturbances.

Thank you.

(The meeting was adjourned)

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Digitalis and Other Cardiotonic Drugs. 2nd Edition. By ELI RODIN MOVITT. 245 pp. $5.75. *Oxford University Press, New York.*

In recent years relatively pure preparations of the cardiac glycosides have become available in quantities sufficient for clinical experimentation and have finally attained an important place in practical therapeutics. The need for a review of the modern literature, particularly that pertaining to the pure glycosides, motivated the author in the preparation of the first edition of this book, which appeared in 1946. The increasing experience in the clinical use of the glycosides and the experimentation on the human subject made possible by the technique of cardiac catheterization have so rapidly added to the pertinent literature as amply to justify the present revision of the work.

The book consists of separate discussions of the various materials of therapeutic importance, the crude botanical preparations as well as the pure principles. In each instance the source and chemistry, laboratory studies, and clinical applications are reviewed. In a field as confused and controversial as that of the physiology of congestive heart failure and the effects of drugs thereon, the author has on the whole achieved sound judgments and lucidity of presentation. This small volume should prove valuable as a key to a voluminous literature and as a guide to the practical clinical use of the numerous glycosidal preparations now available.

T. C. B.

Functional Localization in Relation to Frontal Lobotomy. By JOHN F. FULTON. 140 pp. $3.00. *Oxford University Press, New York.*

The four chapters making up this brief monograph constitute the William Withering Memorial Lectures, delivered by the author at the University of Birmingham in 1948. They are titled: 1) The Frontal Lobes: The Precentral Motor Cortex; 2) The Frontal Areas and the Orbital Surface: Subhuman Primates; 3) The Frontal Areas and the Orbital Surface: Man; and 4) The Cerebellum Reconsidered. An appreciation of William Withering preceeds them; quotations from Sherrington, Ferrier, and Willis are interspersed; an index is appended.

The first lecture deals with the results of ablation and stimulation of the precentral cortex. The author aligns himself with those who believe that muscles rather than movements are represented in the precentral gyrus. He accepts without reservation the existence of multiple suppressor bands in the cerebral cortex, although their role in normal functioning, how they may act, and indeed whether or not they are experimental artifacts are subjects currently very much in question.

The second and third lectures present the main thesis: that the frontal lobes of the brain are principally concerned with the regulation of the autonomic nerv-

ous system, and hence with the peripheral effectors innervated by it. In the author's own words (p. 55): "Indeed, I am inclined to regard the recognition of the frontal lobes as the primary autonomic centre of the forebrain as perhaps the most significant disclosure of recent years in the field of scientific medicine, for it affords at long last a sound physiological background to that still shadowy subject of 'psychosomatic medicine'." It is worthwhile pointing out that the experimental evidence upon which this statement is based is at best tentative, and that final conclusions must await its extension and confirmation. So far as the evidence presented in this monograph is relevant, the psyche and the soma have still only a nodding acquaintance.

The lecture on the cerebellum is of interest chiefly in the report of the work of Nulsen, Black and Drake on inhibition and facilitation of motor activity from the anterior cerebellum. Its full dress publication is eagerly awaited.

Professor Fulton has reviewed briefly here the available information concerning the functions of the frontal lobes of the brain, and has attempted to correlate it with the changes in behavior which follow the procedure of frontal lobotomy, now widely practiced in the treatment of mental disease. The generally dissatisfying result is a measure of the paucity of knowledge of how the brain functions in normal human beings. There is as yet no explanation of how alterations in that function result in mental disease.

Finally the reviewer takes issue on moral and philosophical grounds with the author's defense of frontal lobotomy as an economic measure that will save so much money per patient per day. Like all other therapeutic procedures it must stand or fall on what beneficial effects it may have on the patient, effects which must greatly outweigh the harmful ones which follow such grevious insult to the brain.

V.B.M.

Hemorrhagic Disorders. A Guide to Diagnosis and Treatment. By P. M. AGGELER AND S. P. LUCIA. 112 pp. $10.00. *The University of Chicago Press, Chicago,* 1949.

This is a graphically illustrated monograph on hemorrhagic disorders so written that it presents clearly, though in a very abbreviated manner, certain aspects of the physiology of hemostasis, diagnostic hemostatic tests, and clinical characteristics of hemorrhagic disorders with therapeutic agents and procedures. In the first section the various components participating in blood coagulation and the functions of the platelets and vascular system are briefly considered. Most of the discussion and illustrations on hemostatic tests are devoted to the techniques for the determination of prothrombin time and clot retraction. The statistical analyses of the routine results are worthy of note. In the list of hemorrhagic diseases the bleeding tendency associated with liver damage receives a rather disproportionately large amount of space. When possible the hemostatic defects in each disease are listed. The more common manifestations, the results obtained in various diagnostic hemostatic tests and the treatment of each disease are recorded. The fourth sec-

tion is devoted to a brief description of various therapeutic agents and procedures. The limitations in the use of anti-hemophilic globulin and toluidine blue are not mentioned. A good bibliography is appended. The book fulfills its expressed purpose of presenting certain aspects of the subject of abnormal bleeding in a manner that will be readily understood by the practicing physician, medical student and laboratory technician. It is unfortunate the book is so expensive.

P. F. W.

Metabolism and Function. A Collection of Papers Dedicated to Otto Myerhof on the Occasion of his 65th Birthday. Edited by D. NACHMANSOHN. 348 pp. $7.00. *Elsevier Publishing Co., New York.*

Thirty-four friends, associates and scientific descendants of Otto Meyerhof, have presented a *Festschrift* on the 65th anniversary of his birthday. Five of the contributors are Nobel laureates and many of the others are of equal caliber. Each has written either a speculative and interpretative essay or a precise description of experimentation in the field of his own interest. The extent of these interests is amazingly wide and reflects accurately the scope of Meyerhof's brilliant investigations in so many fields. There are papers on muscle, on nerve and on a variety of enzymatic studies; but through all there runs the linking thread of concentration on the basic problems of energy transformation. This volume is an absorbing portrait of an era in biological thought, an era which has seen the first illumination of those obscure mechanisms which subserve cellular function.

Often anniversary volumes serve mainly to bury work where their contents fail to be included in the conventional indices of literature. This volume, fortunately, appears concurrently as an issue of the journal *Biochimica et Biophysica Acta.*

J. L. L., JR.

Multi-Enzyme Systems. By MALCOLM DIXON. 101 pp. $1.75. *Cambridge University Press, New York.*

This series of four lectures outlines instructively, but very briefly, how groups of enzymes can be coupled functionally into systems which can catalyze energy-yielding reactions and transfer and use this energy for other processes. Of special importance in living matter are two classes of reactions, the so-called hydrogen transfer reactions and the phosphate transfer reactions; and an analogy is drawn between dehydrogenase reactions in which hydrogen or its equivalent is transferred from molecule to molecule *via* coenzyme and phosphokinase reactions in which phosphate groups are transferred *via* adenosine diphosphate. Following this, there is a discussion of the energetics of these reactions, including an attempt at their systematization.

In the treatment of 'hydrogen transfer', no distinction is made between *convenience* of the concept and its experimental reality. Thus, reduced cozymase is referred to as the dihydro-compound which is able to transfer its two hydrogens. Experiment indicates that it has only one transferable hydrogen (a second one comes from the environing buffer), but that it does lose two electrons in the process.

The fact that this misstatement has almost universal currency is a rather curious circumstance.

Moreover, the author compounds the misdemeanor by implying a universal role to *molecular* hydrogen as the agent in biological reductions. He is on presumably safe experimental ground when referring to actual pressures of hydrogen as being in equilibrium with the relatively few systems now known to have a potential at or near that of the hydrogen electrode. However, for the case of the many more systems the potentials of which correspond formalistically to extremely small hydrogen pressures, the author continues in the same vein by asserting that such pressures are quite definite. He informs us, for example, that the quinhydrone electrode is really a hydrogen electrode with the H_2 pressures fixed at 10^{-22} atmospheres. Since this figure corresponds to one molecule of hydrogen in about 3.7 liters, one may well wonder at the physical and chemical significance of such a 'definite' calculation value.

The construct rH, a thermodynamic function of the hydrogen pressure, was decently interred long ago by its originator who saw that its superficial utility was greatly overshadowed by its essential artificiality. Its exhumation in this book seems to be a dubious service, and not in the interests of clear scientific thinking. As a matter of fact, the advantages claimed for the rH system are implicit in the primary electrode data, and can be made equally explicit by a very simple transposition of one coordinate.

Let the reader not be misled by this *caveat*. If certain artificial concepts are reinterpreted, this book is well worth reading and study by all who are interested in enzymes and their mode of function in living matter.

B. C.

Surgery of Repair. 2 Vols. By JOHN F. PICK. 828 pp. $24.00. *J. B. Lippincott Co., Philadelphia, Pa.*

As the author states in his preface, this book is intended for those who are desirous of applying more understandingly the principles, practice and motives of plastic surgery in their daily work.

Approximately one half of the first volume is devoted to an approach to this type of surgery. There is a brief and well-done chapter on the history of the development of general plastic surgery. Several chapters are given to physiological and psychological considerations and the scope and limitations of the subject. The author has attempted fairly successfully to systematize diagnostic problems by classifying them as voids, excesses and derangements, a scheme which is followed throughout the book. The remainder of volume one is devoted to general problems of reconstruction while volume two deals with the special problems of the anatomical divisions of the head and body. One of the best chapters is that on skin grafts and grafting. In this are included also the various types of flaps. The discussion on the grafting of other tissues is good as is also that on the closure of surface defects by "surgical geometrics." In the latter are included all of the time-honored methods designed to close such defects although many might well be

forgotten. The author's method of planning the closure of semicircular and circular defects appears to be original and useful. More critical comment would frequently be desirable as, for example, between the methods for treating an axillary web shown in figures 279 and 280. There is no discussion of the many problems associated with secondary repairs of cleft lip and palate. Hand and major jaw reconstructions merit further attention.

The literary quality of this work is unfortunate. One's attention is far too frequently distracted by linguistic solecisms, barbarisms, and improprieties. Common errors of medical writing are found, such as "temperature" for fever, "pathology" for disease, disorder or pathologic change, "to operate" such cases, and that product of legalistic illiteracy "and/or". But in addition, throughout these volumes, the author has consistently rejected the usual, understandable and explicit word in favor of one which, frequently of his own coinage, may or may not have an applicable meaning. Surely there is no need to pervert "timbre" to designate the textural quality of the skin, nor does "ilk" mean kind or sort. Certain chapters have been "authored". Other random examples of careless usage are staticism, flair, ingenious, local, toilette, desensitization, to edematize, collateral, to ablate, exadurated, decise, lymphstasis, and electronegative.

These volumes are well printed, illustrated, and indexed. Students will find the field of plastic surgery generally well covered while more experienced surgeons will encounter much that is interesting and stimulating.

EDWARD M. HANRAHAN

The Yellow Emperor's Classic of Internal Medicine (*Huang Ti Nei Ching Su Wên*). By ILZA VEITH. 253 pp. $5.00. The Williams and Wilkins Co., Baltimore, Md.

Dr. Veith's translation of the Huang Ti Nei Ching Su Wên, the Yellow Emperor's Classic of Internal Medicine, fulfills an important function by bringing this very influential Chinese medical literature to the occidental world. Though the exact age and origin of the Nei Ching is obscured in ancient legend, it is the basis of Chinese and Japanese orthordox medicine from ancient times up to the present day.

The first part of this book is the introductory study in which Dr. Veith gives us an analysis of the historical background of the Nei Ching and the basic data on the ancient Chinese concepts of religion and philosophy. This introductory study comprises over one-fourth of the book. It is well analyzed and arranged with many excerpts and illustrations put in to help the reader understand the translation easier.

The second part of the book is the actual translation of the Nei Ching. Dr. Veith did not translate the entire work. However, the first thirty-four chapters contain nearly all the basic ideas of this Chinese medical literature. The Nei Ching was written as conversations between the Yellow Emperor and his minister physician, Ch'i Pai. It is divided into many treatises, which deal not only with medical concepts, but also general ethics, religion, philosophy and regimen of life.

The interpretation of old Chinese text is very difficult, not only for Westerners, but also for Chinese scholars as well. Dr. Veith's translation can be read fluently.

The terminology and conceptional content of the Chinese medical and philosophical thought are in general very symbolic and abstract. However, it is very likely that many of them could not be directly translated into the Western language. Dr. Veith's translation is the approach of a medical historian, rather than that of a Chinese philologist.

This book should be welcomed by those who are interested in medical history and Chinese culture, also for those who are interested in the development of scientific medicine in China.

If modern scientific medicine is ever to reach the hinterlands of present-day China, it will be essential to have an understanding of the practices of native Chinese medicine, which is based so heavily on the theories in Nei Ching.

CH'IEN LIU.

BOOKS RECEIVED

Advances in Surgery, vol. 2. Edited by WILLIAM DEWITT ANDRUS. 590 pp. $11.00. *Interscience Publishers, New York.*

Biological Antioxidants. Transactions of the Third Conference, October 7–8, 1948. 146 pp. $2.70. *Publication of the Josiah Macy, Jr. Foundation, New York.*

Blood Clotting and Allied Problems. Transactions of the Second Conference, January 24–25, 1949. 231 pp. $2.25. *Publication of the Josiah Macy, Jr. Foundation, New York.*

Child Hygiene in Baltimore and in Maryland. By J. H. MASON KNOX, JR., Consultant, Bureau of Child Hygiene, Maryland State Department of Health. 36 pp.

Essential Urology. By FLETCHER H. COLBY. 570 pp. $8.00. *The Williams and Wilkins Co., Baltimore, Maryland.*

Lehrbuch der Verdaungskrankheiten. By NORBERT HENNING AND WALTHER BAUMANN. 799 pp. 68 marks. *Georg Thieme Verlag, Stuttgart.*

Metabolic Interrelations. Transactions of the First Conference. Edited by EDWARD C. REIFENSTEIN, JR. 193 pp. $2.95. *Publication of the Josiah Macy, Jr. Foundation, New York.*

The Physiology of Thought. By HAROLD BAILEY. 313 pp. $3.75. *The William-Frederick Press, New York.*

Primer of Allergy, 3rd edition. By WARREN T. VAUGHAN. Revised by J. Harvey Black. 175 pp. $3.50. *The C. V. Mosby Co., St. Louis, Missouri.*

Principles and Practice of Plastic Surgery. By ARTHUR JOSEPH BARSKY. 499 pp. $10.00. *The Williams and Wilkins Co., Baltimore, Maryland.*